



WHITE MATTER FIBRES DISSECTION IN THE HUMAN BRAIN

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ABSTRACT

Introduction: lesion to white matter fibres can induce permanent neurological deficits due to the induction of disconnection syndromes. Knowledge of white matter fibre anatomy is therefore relevant to the neurosurgeon in order to minimise the risk of causing neurological damage when approaching lesions in eloquent areas of the brain.

Aim: to investigate the 3D anatomy of white matter fibres with particular attention to the associative tracts, including short arcuate fibres and intralobar fibres. The results obtained will be used to provide insights in brain connectivity, delineating networks important for specific brain functions.

Methods: The Klingler technique for white matter dissection was followed. Brain specimens were collected and prepared at the Newcastle Brain Tissue Resource, Newcastle University. Brains were initially fixed in 10% formalin for at least 4 weeks. After removing the pia-mater and arachnoid, the brains were frozen at -15C° for 2 weeks. The water crystallisation induced by the freezing process separates the white matter fibres, facilitating the dissection of the tracts. Dissection was performed with wooden spatulas and blunt metallic dissectors, removing the cortex and exposing the white matter. The short associative (U-shaped) fibres were initially exposed. Long associative, commissural and projection fibres were demonstrated as the dissection proceeded.

Results: five papers form the main body of the present work:

- 1) *“Raymond de Vieussens and his contribution to the study of white matter anatomy”*. This historical paper reviewed the history of white matter dissection, focusing on the work of Raymond de Vieussens, who gave the first account of the centrum ovale and of the continuity of the corticospinal tract from the centrum ovale to the brainstem.
- 2) *“The white matter of the human cerebrum: part I The occipital lobe by Heinrich Sachs “;*
- 3) *“Intralobar fibres of the occipital lobe: A post mortem dissection study”*. These joint papers were dedicated to the white matter anatomy of the occipital lobe. A rich network of association fibres, arranged around the ventricular wall, was demonstrated. A new white matter tract, connecting the cuneus to the lingula, was also described. Our original data

were compared to the atlas of occipital fibres produced by the German anatomist Heinrich Sachs.

4) *“White matter connections of the Supplementary Motor Area (SMA) in humans”*. This study demonstrated that the SMA shows a wide range of connections with motor, language and limbic areas. Features of the SMA syndrome (akinesia and mutism) can be better understood on the basis of these findings.

5) *“Anatomical connections of the Subgenual Cingulate Region” (SCG)*. This study showed that the SCG is at the centre of a large network, connecting prefrontal, limbic and mesotemporal regions. The connectivity of this region can help explain the clinical effect of neuromodulation of the SCG in Deep Brain Stimulation for neuropsychiatric disorders.

Conclusions: Klingler dissection provided original data about the connections of different brain regions that are relevant to neurosurgical practice, along with the description of a new white matter tract, connecting the cuneus to the lingula.

Acknowledgments

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- *Chiara my wife, and my two boys Pietro and Federico: for all the love and joy they bring in my life*

List of abbreviations

AF: Arcuate Fasciculus

Cing: Cingulate gyrus

DBS: Deep Brain Stimulation

DTI: Diffusion Tensor Imaging

FAT: Frontal Aslant Tract

IFOF: Inferior Fronto-Occipital Fasciculus

ILF: Inferior Longitudinal Fasciculus

IFG: Inferior Frontal Gyrus

MFG: Middle Frontal Gyrus

MRI: Magnetic Resonance Imaging

PreCG: Precentral Gyrus

PoCG: Postcentral Gyrus

SCG: Subgenual Cingulate Gyrus

SFG: Superior Frontal Gyrus

SLF: Superior Longitudinal Fasciculus

SMA: Supplemenatry Motor Area

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Chapter 1. Introduction and Aim

1.1. Introduction

The white matter is the part of the brain which contains the axons of neurons. It is organized in fibre tracts (also known as fascicles or bundles) that connect different parts of the brain and contains the efferent and afferent pathways from and to the cortex. Three different types of fibres have been described (Rothon 2002):

- **associative fibres**, linking different regions within the same hemisphere. These are further differentiated in short or arcuate “U-fibres”, that connect neighbouring gyri and long associative fibres, that connect distant regions usually located in different lobes of the hemisphere;
- **commissural fibres**, that cross the midline, connecting homologous regions of the two hemispheres. Example of commissural fibres are the corpus callosum, the anterior commissure and the hippocampal commissure;
- **projection fibres**, that connect the cortex with the diencephalon, brainstem, cerebellum and spinal cord. Example of projection fibres are the cortico-spinal tract and the thalamo-cortical projections.

Knowledge of the white matter is important to delineate the subcortical connectivity of the human brain. This is of relevance to the understanding of brain networks subserving specific functions. Recent studies in the literature have focused on the role of white matter connectivity in language, visuospatial attention, complex motor functions and memory (Catani 2005, Duffau 2008, Holodny 2005, Urbanski 2008). Involvement of white matter structures is also of emerging interest in elucidating the mechanisms of cognitive decline in ageing and neurodegenerative disorders (Chua 2008, Sullivan 2006). From a clinical

perspective, lesions involving white matter connections are relevant as they can cause a “disconnection syndrome” (Catani, 2008a). The first example of disconnection syndrome was described by Dejerine in the XIX century, with a case of “alexia without agraphia” in a patient with a lesion involving the splenium of the corpus callosum and the occipital lobe. The patient of Dejerine was able to write and retained a normal spoken language, but was unable to read; this reflected a disconnection between visual and language areas. The concept of disconnection syndrome was further developed in the XIX century thanks to the work of Geschwind (Geschwind 1965a, Geschwind 1965b). According to Geschwind, an alteration of cognitive functions can occur due to the disruption of the white matter connections within a network. Therefore, in a disconnection syndrome, the function of different and distant cortical regions can be affected when the fibres connecting these regions are damaged (Catani and ffytche, 2010). Of interest, the individual function of each region can still be preserved; it is the function obtained from the interaction between these regions that is affected (figure 1).

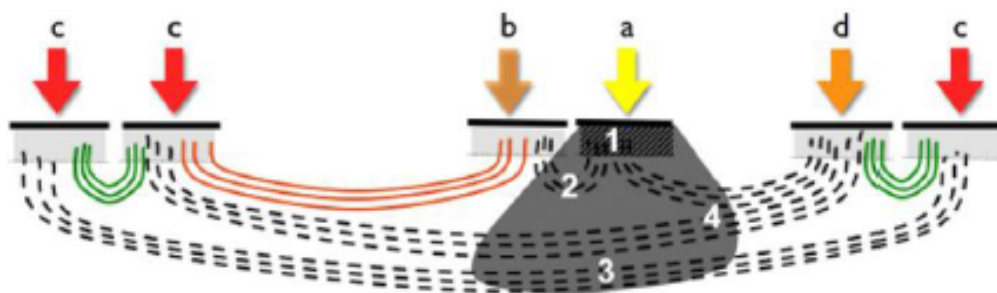


Figure 1. Schematic representation of the mechanisms underlying a disconnection syndrome. A lesion involving the cortex and the subcortical white matter (e.g., a tumour) has been represented in gray. The cortical area “a” is directly affected by the lesion (number 1). The function of area “b” is affected by damage to the short U-fiber connections between “a” and “b” (number 2). The function of areas “c” is affected by lesion of long associative fibers (number 3). Finally, area “d” is affected by lesion of both short and long associative fibers (number 4). (Figure taken from Catani and ffytche, 2010)

To the neurosurgeon, knowledge of the white matter anatomy is important to refine surgical approaches to brain lesions such as tumours, haematomas or vascular malformations when these are located in eloquent areas (Fernandez-Miranda 2008, Martino 2010a). Numerous articles in the neurosurgical literature have focused on the importance of sparing the white matter tracts. Surgical approaches to lesions in close proximity to the primary motor area can affect the cortico-spinal tract, causing an irreversible motor deficit (Berger 1995, Keles 2004, Kamada 2005). Surgery in the temporal lobe can damage the optic radiations, particularly at the level of the Meyer's loop around the ventricle, causing a contralateral quadrantanopia (Peltier 2006, Nilsson 2007). Surgery in the perisylvian region can induce language deficits, with conduction aphasia due to a lesion of the arcuate fasciculus (Duffau 2002, Martino 2013). These examples demonstrate how a detailed understanding of the 3-D relationship of white matter fibres is of paramount importance in neurosurgery to prevent neurological damage and improve surgical outcome.

Despite the advent of Diffusion Tensor Imaging (DTI) tractography, that allows the study of white matter tracts *in vivo* (Catani 2002, Catani 2008b), there is still controversy about the anatomy of some of the major tracts, particularly regarding the cortical termination of individual fibres. This is mainly due to technical limitations of the DTI, which has a limited resolution in following the fibres to their cortical termination and also in discriminating between different fibres tracts when they run in close proximity to each other. The post-mortem dissection technique originally developed by Klingler (Klingler 1935) is a well established method to investigate the anatomy of the white matter (Ture 2000). It can potentially overcome the limits of DTI, thus adding valuable information to radiological studies.

1.2. Aim of the study

The aim of the present study is to use the Klingler dissection technique to investigate the 3-D anatomy of white matter tracts. Particular attention will be dedicated to the associative tracts, including short arcuate fibres and intralobar fibres, which have so far received less attention in the literature, but that can play an important role in brain connectivity (Catani 2012).

The results obtained have numerous applications, including:

- refine surgical approaches to brain lesions in eloquent areas. The course of white matter tracts can be compared with results from studies where subcortical stimulation is applied, to find correspondence between the anatomy and the function of individual tracts. This has important clinical implications, in avoiding neurological deficits.
- provide insights in brain connectivity, delineating networks important for specific brain functions.
- compare *post-mortem* findings obtained from dissections with *in vivo* data from DTI. The two techniques can in this way validate each other, at the same time corroborating the anatomical findings. Any discrepancy between the findings obtained with the two methods will be discussed and further studies can be planned to elucidate these differences.

Chapter 2. Methods

Brains are collected from donors who have expressed in life their wish to donate their brain after death. The specimens are collected and prepared at the Newcastle Brain Tissue Resource, Institute for Ageing and Health, according to the Human Tissue Authority regulations. The Klingler method for study of the white matter is followed (Klingler 1935).

Brains are initially fixed in 10% formalin for at least 4 weeks. Once the fixation process is completed, the pia-mater and arachnoid are removed (figure 2). The brains are then frozen at -15C° for 2 weeks. The water crystallization induced by the freezing process disrupts the structure of the gray matter (which has a high water content), thus making it easier to peel off the cortex from the brain surface. The freezing process also separates the white matter fibres, facilitating the dissection of the fibre tracts. (figure 3). After the completion of the freezing process, the dissection is started. A detailed description of the superficial anatomy of cortical gyri and sulci is performed. Dissection is then carried out with wooden spatulas and blunt metallic dissectors, removing the cortex and exposing the white matter. The short associative (U-shaped) fibers are initially exposed. As the dissection proceeds deeper, long association and commissural fibers can be demonstrated (figure 4). Digital photographs are taken to document the stages of the dissection and the most relevant findings. The cortical origin, course and relationships of the fibres are described, with particular attention to all information relevant to neurosurgical anatomy.

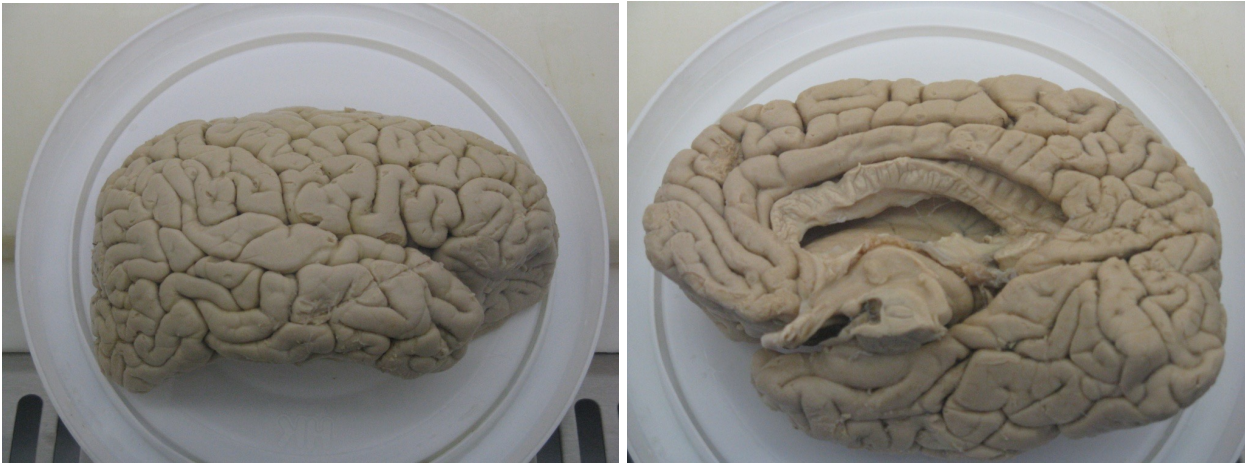


Figure 2. Brain specimens after removal of the pia-arachnoid. Lateral aspect of right hemisphere (left) and medial aspect of the same hemisphere (right).

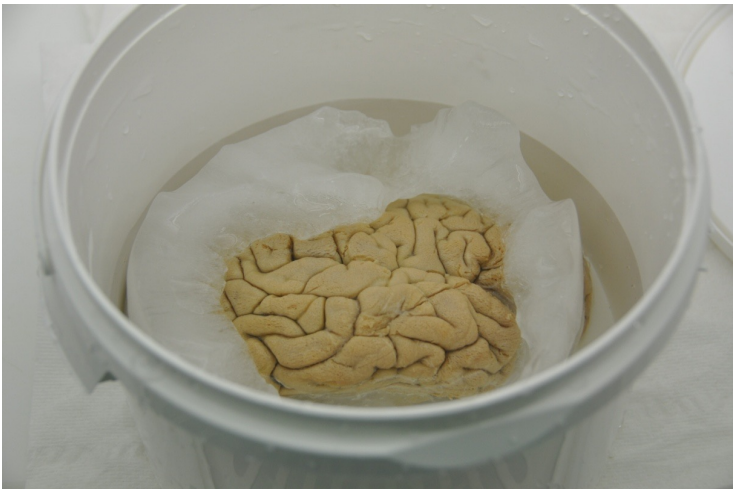


Figure 3. Brain frozen at -15° for 2 weeks. The freezing process facilitates the dissection of white matter tracts.

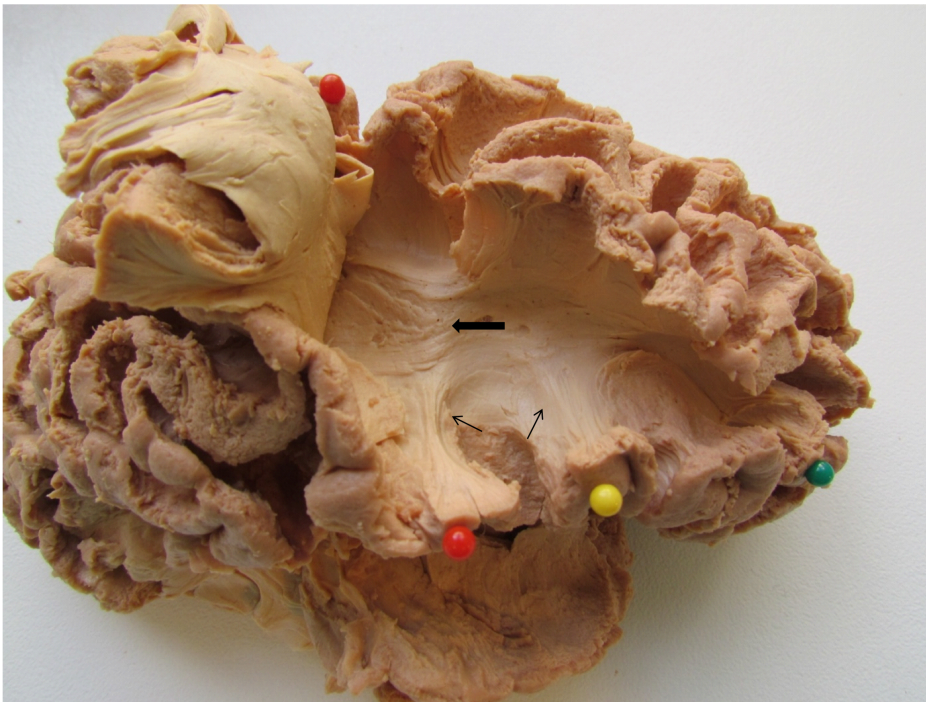


Figure 4. Example of dissection of white matter fibres. Lateral view of a right hemisphere. The middle frontal gyrus has been partially removed, with exposure of short U fibers connecting neighbouring circonvolutions of the inferior frontal gyrus (small arrows). Deeper to the middle frontal gyrus a long associative tract, the Superior Longitudinal Fasciculus (large arrow) has been exposed.

Chapter 3. Results

3.1. First paper – “Raymond de Vieussens and his contribution to the study of white matter anatomy: historical vignette”

Francesco Vergani, Chris Morris, Patrick Mitchell, Hugues Duffau

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This paper provides an historical background to the present research. It was important to acknowledge and highlight the contributions provided by prominent neuroanatomists from the past, with their early difficulties in developing optimal techniques for white matter dissection and the interpretation given by them to the anatomical findings, at a time when neurophysiology was still primordial. This put my research into an historical context, to remember that if nowadays we have a better knowledge and understanding of neuroanatomy (and neuroscience in general) it is simply because we are standing “on the shoulders of giants”.

The article focuses on the life and work of Raymond de Vieussens, a XVII century French anatomist, who gave a major contribution to the study of white matter anatomy. His main work, the “Neurographia Universalis”, still considered one of the most complete and accurate description of the nervous system provided in the XVII century, gives the first account of the centrum ovale and of the continuity of the white matter fibres from the centrum ovale to the brainstem.

3.1.1. Historical background

Andreas Vesalius (1514-1564), the father of modern anatomy, was the first to differentiate between the gray and white matter of the brain. In his main work, *De humani corporis fabrica*, he distinguished between the softer and yellowish cortex and the firmer, whiter substance below it. He also described in detail the corpus callosum and recognized that it connected the two hemispheres. We have to wait a century after Vesalius before the first attempts to dissect and investigate in detail the anatomy of the white matter were made (Schmahmman and Pandya 2009). Nicolaus Steno (1638-1686) suggested for the first time to follow “the threads through the substance of the brain to find out where they go and where they end” (Clarke and O’Malley 1996). One of the problems at the time was the preparation of the cadaveric specimens, as dissection of the white matter was technically difficult. This was partially overcome by Marcello Malpighi (1628-1686). Boiling the brains in water, he was able to trace the white matter bundles of the brain and cerebellum. He was also the first to show, using a microscope, that the white matter was organized in fibres. After Malpighi, Thomas Willis (1621-1675) working in Oxford developed a technique of scraping the white matter to demonstrate the bundles and the intricate arrangements of the fibres.

3.1.2. Raymond de Vieussens and the *Neurographia Universalis*

Raymond de Vieussens was born in 1641 in the city of Le Vigan in southern France (Podolski 1952, Dulieu 1967, Loukas 2007). He studied philosophy at Rodez in France before moving to Montpellier where he embraced anatomical research and medicine. He completed his studies and graduated in medicine from the University of Montpellier in 1670. After graduation he was appointed as physician to the Saint-Eloi hospital in

Montpellier. Here he devoted “ten years of hard work” (*decem annorum labor improbus*) to the study of the nervous system; this work eventually led to the publication of the *Neurographia Universalis* in 1684. This first work was well accepted by the European medical community as one of the most complete descriptions of the nervous system of his time. It also brought Vieussens recognition both in France (where he was appointed member of the Academy of Sciences of Paris) and abroad (he became fellow of the Royal Society of London). During his late years Vieussens continued to work at Saint-Eloi hospital, where he developed an interest in the study of heart anatomy and cardiology.

The “*Neurographia Universalis, hoc est omnium corporis humani nervorum, simul et cerebri, medullaeque spinalis descriptio anatomica*” (General Neurography, i.e. the anatomic description of all the nerves of the human body, and also of the brain and spinal cord) was published in Toulouse in 1684 by the editor Joannes Certe (figure 1.1). The treatise is divided in three parts: the first volume is dedicated to the brain, the second to the spinal cord and the third to the nerves (both intracranial and peripheral). From a methodological point of view, Vieussens adopted the scraping method of dissection used by Willis, and improved the technique of Malpighi of boiling the brains, using oil instead of water (Schmahmann and Pandya 2009). In chapter X of the first volume, “*De distinctis duabus cerebri substantiis*” (“the two distinct substances of the brain”) Vieussens clearly differentiates between white and grey matter highlighting the different texture between the two. In chapter XI, “*De cerebro stricte sumpto*” (“the brain considered in strict sense”) Vieussens describes the two hemispheres and the convexity of the brain. He gives a detailed account of the corpus callosum, identifying it as a white matter structure connecting the two halves of the brain. He then illustrates the *centrum ovale*, the oval-shaped white matter lying beneath the cortex and surrounding the corpus callosum and the ventricular walls, as demonstrated in “tabula VI” of the first volume (figure 1.2). The

introduction of the term *centrum ovale* is one of the legacies to the field of neuroanatomy left by Vieussens. The method followed by Vieussens in his description of the brain anatomy is of a “top to bottom” dissection: starting from the plane of the centrum ovale, he pursues the dissection inferiorly, exposing the lateral ventricles (with the foramen of Monro, called by Vieussens *vulva*), the septum pellucidum, the fornix, the third ventricle and the thalami. Following the fibers caudally Vieussens was able to demonstrate, for the first time, the continuity of the white matter through what is known today as the internal capsule, down to the pyramidal tracts and the brainstem (figure 1.3).

Despite this accurate and precise anatomical description, the physiological explanation provided by Vieussens was still strongly influenced by the theories of the “animal spirit” of Galen. In Vieussens’ view, the structural organisation of the white matter fibers, running from the centrum ovale to the medulla oblongata and the spinal cord, has the purpose of conveying the animal spirit. “The white matter is formed by long and curved fibers, that are so mixed and interrelated that they take the form of a spongy body, that the animal spirit permeates in multiple, different and inexplicable ways; so that within it (the spirit) undergoes multiple, different and inexplicable emotions; because of their different arrangements, different thoughts are generated in the mind”.

3.1.3. The legacy of Raymond de Vieussens

The *Neurographia Universalis* had a great impact on the study of neuroanatomy at Vieussens' time. Many investigators continued Vieussens' work. With a technical improvement, obtained by hardening the brain in an alcohol solution, Felix Vicq d’Azir (1748-1794) confirmed in his dissections many of Vieussens' findings. D’Azir also provided a more detailed description of the centrum ovale, that he renamed “centrum semiovale”.

More than a century after Vieussens' work, Johann Christian Reil (1759-1813) introduced the term "corona radiata" to describe the centrum ovale. He clarified the relationship and the continuity between the fibers of the centrum ovale and the internal capsule and cerebral peduncles, at the same time describing the insula and the external capsule. Relatively few authors were involved in the study of white matter pathways during the following centuries, notably Karl Friedrich Burdach (1776-1847), Pierre-Louis Gratiolait (1815-1865) and Joseph Jules Dejerine (1849-1917). Joseph Klingler (1888-1966) an anatomical preparator at the University of Basel, developed the current standard for white matter dissection in post-mortem human specimens, introducing the process of freezing previously fixed brains (Agrawal 2011, Klingler 1935). The Klingler method allowed for more accurate anatomical investigations, with the clear identification of the major white matter fascicles (Ture 2000, Catani 2012, Choi 2006, Mahaney 2008, Martino 2010b, Martino 2011).

Raymond de Vieussens opened the door to investigating the white matter of the brain in men. His pioneering work showed that the white matter is not an homogeneous and indistinct substance, but rather a complex structure, rich of fibres that are interconnected with different parts of the brain. These initial results paved the way to the advancements observed in the following centuries that have subsequently found application in so many fields of neuroscience.

RAYMUNDI
VIEUSSENS
DOCTORIS MEDICI
MONSPELIENSIS
NEUROGRAPHIA
UNIVERSALIS.

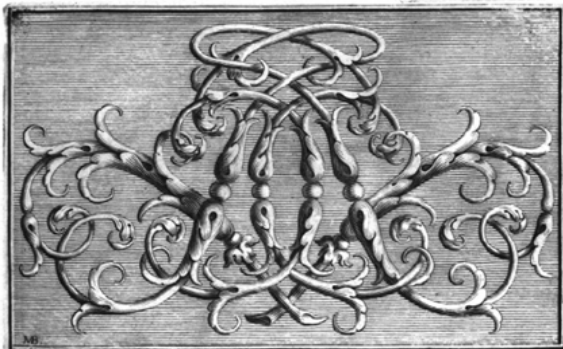
HOC EST,
OMNIUM CORPORIS HUMANI NERVORUM,
simul & cerebri, medullæque spinalis

DESCRIPTIO ANATOMICA;

EAQUE INTEGRA ET ACCURATA, VARIIS
Iconibus fideliter & ad vivum delineatis, æque incisís illustrata:

CUM IPSORUM ACTIONE ET USU,
Phyfico discursu explicatis.

EDITIO NOVA.



LUGDUNI,

Apud JOANNEM CERTE, in vico Mercatorio,
sub signo Trinitatis.

M. DC. LXXXIV.
CUM PRIVILEGIO REGIS.

Figure 1.1 cover of the Neurographia Universalis (From Neurographia Universalis, 1684, originally published by Joannes Certe)

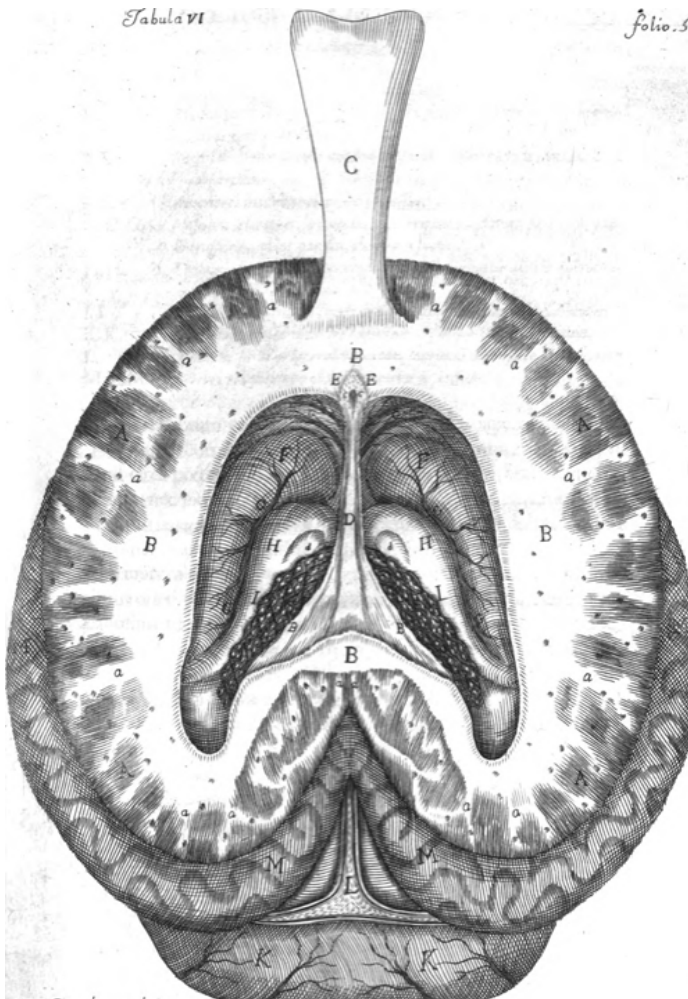


Figure 1.2: tabula VI from the *Neurographia Universalis*. The human brain is sectioned following the “top to bottom” approach used by Vieussens. The gray matter (A) is differentiated from the white matter of the centrum ovale (B), surrounding the ventricles, clearly represented. The corpus callosum (C), sectioned and displaced forward, is also shown.

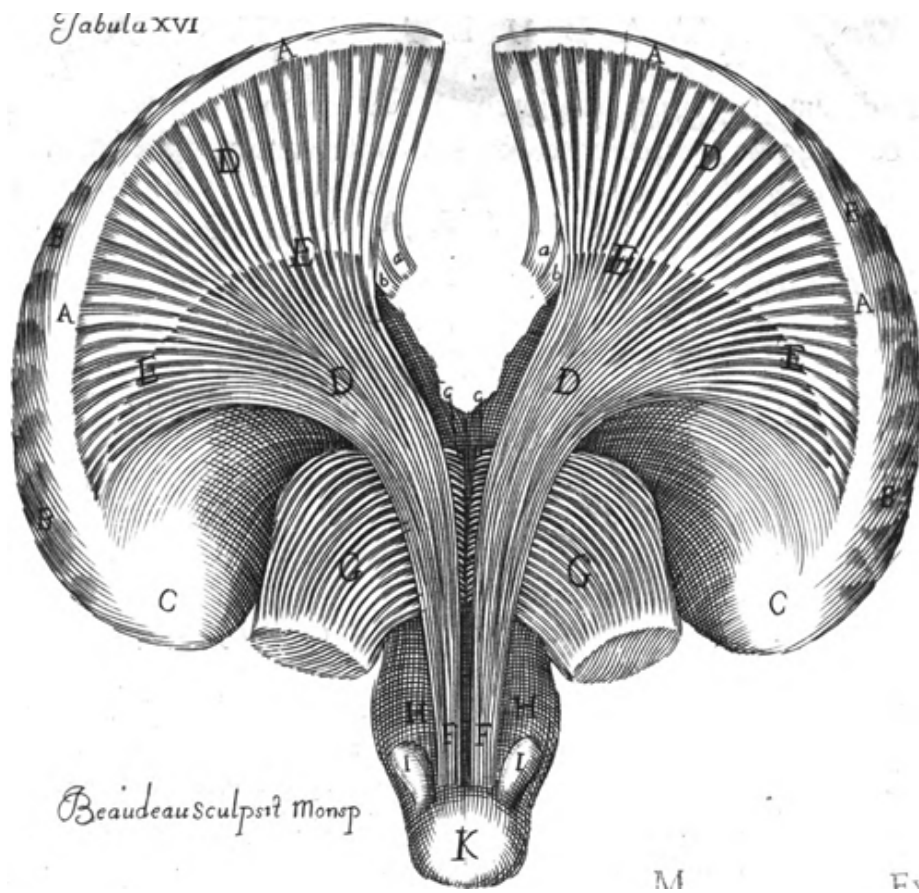


Figure 1.3: tabula XVI from the *Neurographia Universalis*. The continuity of the white matter from the centrum ovale (A) through the *medulla oblongata* (D) is demonstrated.

3.2. Second paper - Intralobar fibres of the occipital lobe: A post mortem dissection study

Francesco Vergani, Sajedha Mahmood, Cristopher Morris, Patrick Mitchell and Stephanie Forkel

Status: published on **Cortex**. 2014 Jul;56:145-56 (Impact Factor: 6.04)

Third paper - The white matter of the human cerebrum: part I The occipital lobe by Heinrich Sachs

Stephanie Forkel, Sajedha Mahmood, Francesco Vergani, Marco Catani

Status: published on **Cortex**. 2015 Jan;62:182-202 (Impact Factor: 6.04)

Traditionally, the white matter anatomy of the occipital lobe has received little attention in the literature. This is probably because the occipital lobe, apart from the primary visual cortex with the optic radiations, was seen as a “non-functional” region of the brain.

Indeed, the most accurate description of the occipital lobe white matter was provided by the atlas published by Heinrich Sachs, a German neurologist and anatomist, in 1892 (Sachs 1892) (see figure 2.1).

These two papers form a joint project that was submitted to Cortex. In the first article we provided original data about the Klingler dissection of three hemispheres, comparing our findings with the description of white matter tracts originally provided by Sachs. In the second article, we performed the first complete English translation of the atlas of Sachs along with a brief description of his life and academic achievements.

A rich network of short association fibres, arranged around the ventricular wall, was demonstrated in the occipital lobe. This comprised U fibres connecting neighbouring gyri and intralobar associative fibres. A new white matter tract, connecting the cuneus to the lingula, was also described.

Lateral to the ventricles, longitudinal fibres of the stratum sagittale were also identified, organized in an external and an internal layers. The importance of the stratum sagittale of the occipital lobe has been recently emphasized by Viegas et al. (Viegas 2011). In their case series of patients operated for WHO grade II gliomas involving the occipital lobe, semantic paraphasias were induced after intraoperative stimulation of the stratum sagittale. This tract constituted therefore the antero-lateral margin of resection, preserving language function in patients undergoing an occipital lobectomy.

3.2.1. Results

Surface anatomy of the occipital lobe

On the medial surface of the hemisphere, two prominent sulci defining the anatomy of the occipital lobe were identified in all three hemispheres (figure 2.2a). The parietoccipital sulcus was recognised at the boundary between the parietal and occipital lobe. This sulcus appeared to be uniformly uninterrupted (Ono 1990) and separated the precuneus (parietal lobe) from the cuneus (occipital lobe). The calcarine fissure, running from the occipital pole towards the splenium of the corpus callosum, divided the cuneus (superior to the fissure) from the lingual gyrus (inferior to the fissure). Both these sulci appeared to be rich in connecting gyri found in the deepest portion of the sulcus. Sachs compared these “gyri brevi” to the insular gyri found in the depth of the Sylvian fissure (figure 2.7a).

On the lateral surface of the brain, the occipital lobe appeared to be in continuity with the posterior part of the parietal and temporal lobes (figure 2.2b). On the dorsal edge of the lateral aspect, the outer margin of the parietooccipital sulcus was identified. Three horizontal sulci were observed on the convexity of the occipital lobe. The transverse occipital sulcus (called by Sachs *sulcus occipitalis superior*, s.o. I) lies lateral to the superior margin of the hemisphere and represents ideally the continuation – in the occipital lobe – of the intraparietal sulcus. The lateral occipital sulcus (*sulcus occipitalis medius*, s.o. II in Sachs'atlas) and the inferior occipital sulcus (*sulcus occipitalis inferior*, s.o. III) lie approximately at the level of the superior and inferior temporal sulci, respectively. These three horizontal sulci identify three gyri on the lateral surface of the occipital lobe: superior, middle and inferior. It has to be noted (as was also recognised by Sachs himself) that the lateral aspect of the occipital lobe shows a great variability in the pattern and presentation of sulci and gyri, with in particular the lateral and inferior sulci being less consistent (“usually interrupted and branched and therefore less clear”). The three gyral pattern described here was recognised in two of our three specimens, while in one specimen only two lateral gyri were found (the inferior sulcus not being present). This variability has been also reported for larger cohorts in contemporary studies (Iaria and Petrides 2007, Iaria 2008, Alves 2012).

On the basal surface of the brain, the occipital lobe continued with the temporal lobe. The more prominent sulcus at this level was the collateral sulcus that divided the lingual gyrus (medially) from the fusiform gyrus (laterally). More laterally, the occipito-temporal sulcus divided the fusiform gyrus (medially) from the inferior temporal gyrus.

Short association fibres

On the medial surface of the brain, dissection of the parietoccipital sulcus showed the presence of U-fibres running in the depth of the sulcus and connecting the superior margin of the cuneus with the inferior aspect of the precuneus (figure 2.7b). Dissection of the calcarine fissure demonstrated a large and relatively thick bundle of U fibres connecting the upper and lower edges of the calcarine cortex (figure 2.7c). Coronal sections performed behind the junction between the parietoccipital sulcus and the calcarine fissure clearly demonstrated U fibres running between the upper and lower bank of the calcarine fissure, too (figures 2.4, 2.5 and 2.6). These fibres correspond to the *stratum calcarinum* described by Sachs. Dissection of the medial aspect of the calcarine fissure demonstrated also a bundle of fibers previously not described by Sachs. This fascicle runs from the inferior portion of the cuneus to the superior and anterior aspect of the lingula, deep to the calcarine fissure (figure 2.7). The bundle presents a supero-inferior and anterior curvature. Because of its peculiar shape, it has been named the “sledge runner” fascicle.

After removing the cortex of the cuneus, U fibres running from the superior to the inferior portion of the cuneus (i.e. to the superior aspect of the calcarine fissure) were exposed (figure 2.7d). These fibres were again confirmed on the coronal sections (figure 2.5). This bundle of fibres corresponds to the *stratum proprium cunei* of Sachs.

On the coronal sections, a bundle of fibres originating at the level of the cuneus and running inferiorly and laterally, was also observed (figure 2.5 and 2.6). This tract was directed towards the lateral wall of the occipital horn, coursing lateral to the stratum sagittale (see below) before pointing more laterally, towards the convexity of the occipital lobe. This bundle corresponds to the *stratum transversum cunei* (transverse stratum of the cuneus) described by Sachs. It was unfortunately not possible to follow the bundle until its

cortical terminations, as it was difficult to differentiate from the outer layer of the stratum sagittale medially and from the fibres of the convexity laterally.

Dissection of the lateral surface of the occipital lobe demonstrated a continuous system of U fibres connecting neighbouring circonvolutions. Thin U fibres were observed lying deep to the cortex of the superior, middle and inferior occipital sulci (figures 2.4, 2.5, 2.6 and 2.8). Sachs named these fibres collectively as “*stratum verticale convexitatis*” (vertical stratum of the convexity), naming each individual fibre after the sulcus containing it (e.g.: *stratum proprium sulci occipitalis I*, and so on). Deeper to this superficial layer of U fibres, a vertical bundle running from the superior to the inferior region of the occipital lobe was encountered (figure 2.5 and 2.8). This tract, rather thin and running lateral to the stratum sagittale, corresponds possibly to the *stratum profundum convexitatis* of Sachs. In our dissections it was difficult to follow this bundle for its entire course, as it appeared to blend with the U fibres of the convexity.

At the basal surface of the occipital lobe, a well defined U fibre tract, running deep to the sulcus collateralis and connecting the gyrus fusiformis with gyrus lingualis was demonstrated (figures 2.4, 2.5 and 2.6). This was the *stratum proprium sulci collateralis* identified by Sachs.

Stratum sagittale

A dense bundle of fibres with a sagittal course and extending from the temporal to the occipital lobe was clearly demonstrated in all coronal sections. This represented the *stratum sagittale* described by Sachs (figures 2.4, 2.5 and 2.6). Fibres of the stratum sagittale are contained between the white matter of the convexity and the ventricle. They extend in a “C” shaped layer embracing the superior, lateral and inferior walls of the

occipital horn of the ventricle. Continuing the dissection of the white matter from lateral to medial, two layers of the stratum sagittale were identified; these two layers were named *stratum sagittale internum* (internal layer) and *stratum sagittale externum* (external layer) by Sachs (figure 2.8). The two layers are contiguous and they are more difficult to appreciate on coronal sections, and in most of our cuts they were not readily identifiable as distinct. The two layers can be better appreciated after carefully separating the *stratum externum* from the *stratum internum* with fine blunt dissectors at the level of the lateral and superior wall of the occipital horn. A distinction between the two layers at the level of the floor of the ventricle was not possible in our study.

Callosal fibres

On coronal sections, fibres surrounding the upper, lateral and medial portion of the occipital horn of the ventricle were also identified. These fibres were situated medially to the above described stratum sagittale, representing the layer of white matter directly in contact with the walls of the ventricle. These fibres correspond to the forceps major, the radiation of commissural fibres arising from the splenium of the corpus callosum and connecting the cortex of the two occipital lobes. Fibres of the forceps were more evident at the level of the splenium of the corpus callosum (figure 2.3). After leaving the splenium, these fibres take an inferior course to run laterally to the lateral wall of the ventricle, at the level of the trigone, taking the name of *tapetum*. More posteriorly, as the occipital horn of the ventricle decreases in size, callosal fibres appeared less well differentiated from the surrounding white matter. At this level, they were nonetheless recognisable, particularly at the superior and medial portion of the ventricular walls (figures 2.4 and 2.5).

3.2.2. Discussion

In the present study we investigated the intralobar white matter fibres of the occipital lobes preparing three hemispheres according to the Klingler's method. We performed coronal sections of the hemispheres as described by Sachs (Sachs 1892), in order to compare our findings with the original description provided in Sachs' atlas. Overall, a striking concordance was observed between the two techniques. All the major tracts described by Sachs were clearly identified in our specimens. The small differences observed in those coronal sections where not every single fibre could be identified may be explained on the basis of the "law of variability", so clearly expressed by Sachs in the introduction to his Atlas: *"There are no two brains that are identical in all their details. This variability is also observed in the order and development of the fibre systems. The cortex and the white matter fibres are dependent on each other. If a particular area of cortex is under-developed in a brain, then there is also a paucity of fibres originating from this area"*. It is therefore possible that small differences exist, particularly at the level of short arcuate fibres. This may also be reflected by the fact that a coronal cut performed at the same level can show a certain amount of fibres in one specimen and other fibres in another.

Two fascicles, although clearly visualised, were particularly difficult to follow with the Klingler's technique. The first is the "transverse stratum of the cuneus", the tract that runs from the cuneus in a medial to lateral direction, curving lateral to the stratum sagittale and then pointing towards the convexity. Particular uncertainty persists regarding the termination of this fascicle at the level of the convexity, that could not be identified with the dissection performed in the present study. The main difficulty encountered was to separate this bundle from the stratum sagittale medially and from the fibres of the convexity laterally. At this level in particular the dissection was rendered difficult due to fibre crossing between the "transverse stratum of the cunes", that shows an horizontal direction, and the "stratum

proprium convexitatis”, that has instead a vertical course. This problem was also encountered by Sachs.

The second bundle was the “stratum profundum convexitatis” (deep layer of the convexity). Although identified as a contingent of vertical fibres running from the superior aspect of the lobe to its inferior surface, this layer of white matter did not appear entirely separated from the more superficial U fibres of the convexity. It is therefore questionable if this “stratum profundum convexitatis” should be considered as an independent white matter tract. Schmahmann and Pandya, in their work on the monkey brain, were also able to identify fibres that link the dorsal and ventral aspects of the occipital lobe, but considered them as being part of a vertical component of the Inferior Longitudinal Fasciculus (ILF) (Schmahmann and Pandya 2006).

Other differences between the present work and Sachs’ atlas were found at the level of the stratum sagittale. According to Sachs, the stratum sagittale is divided in an external layer, that conveys corticopetal fibres from the thalamus to the occipital lobe, and an internal segment that conveys corticofugal fibres from the cortex to the thalamus. In Sachs’s view, the “stratum sagittale internum” is therefore a projection system, while the “stratum sagittale externum” is an associative bundle, connecting the occipital lobe exclusively with the temporal lobe. In our dissections, it was possible to differentiate between the two layers only on the superior and lateral aspect of the ventricular wall. At the level of the floor of the ventricle a thick bundle of white matter, deep to the collateral sulcus, was encountered, with no clear distinction between an external and internal layer. Recent studies, both with post-mortem dissections and DTI, have demonstrated the presence in the occipital lobe of a long association fascicle that runs along the superior and lateral portion of the ventricular wall, lateral to the optic radiations, before reaching its cortical terminations at the level of the occipital, parietal and basotemporal lobes. This fascicle

corresponds to the Inferior Fronto Occipital Fasciculus (IFOF), originally described by Curran (Curran 1909, Martino 2010b, Fernandez-Miranda 2008, Catani 2002). The white matter encasing the inferior wall and the floor of the ventricle, could correspond to the Inferior Longitudinal Fasciculus (ILF), a bundle connecting the temporal and the occipital lobe, and running inferolaterally to the optic radiations and the ventricles (Catani 2003, Fernandez-Miranda 2008). The importance of the stratum sagittale of the occipital lobe has been recently emphasized by Viegas et al. (Viegas 2011). In a retrospective review of patients operated for WHO grade II gliomas involving the occipital lobe, semantic paraphasias were induced after intraoperative stimulation of the IFOF (for lesions located in the dominant hemisphere). This tract constituted therefore the antero-lateral margin of resection, preserving language function in patients undergoing an occipital lobectomy.

One last but relevant difference was observed in the dissection of the medial aspect of the calcarine fissure. As reported by Sachs, “U fibres” connecting the superior and inferior edges of the fissure were clearly demonstrated in our dissections. Unlike Sachs, we identified another fascicle running anteriorly from the inferior portion of the cuneus to the supero-anterior portion of the lingula. This fascicle, due to its peculiar shape, has been named the “sledge runner”. It is likely that Sachs missed the fascicle because in his original work only coronal sections were performed. The “sledge runner” is a thin fascicle, with a postero-anterior course in the sagittal plane, and can therefore be either lost or confused with the other U fibers running in the calcarine fissure in the coronal plane. Our findings represent the first demonstration of this fascicle in post mortem human specimen.

3.2.3. Conclusions

The occipital lobe shows a rich and complex network of intralobar fibres, arranged around the ventricular wall. Short association fibres appear to be superficial, while fibres of the stratum sagittale and of the forceps major are located deeper, in contact with the walls of the ventricle. Good concordance was observed between the Klingler dissection technique and the histological preparations of Sachs. The “sledge runner” fascicle, connecting the cuneus to the lingula, was also demonstrated.

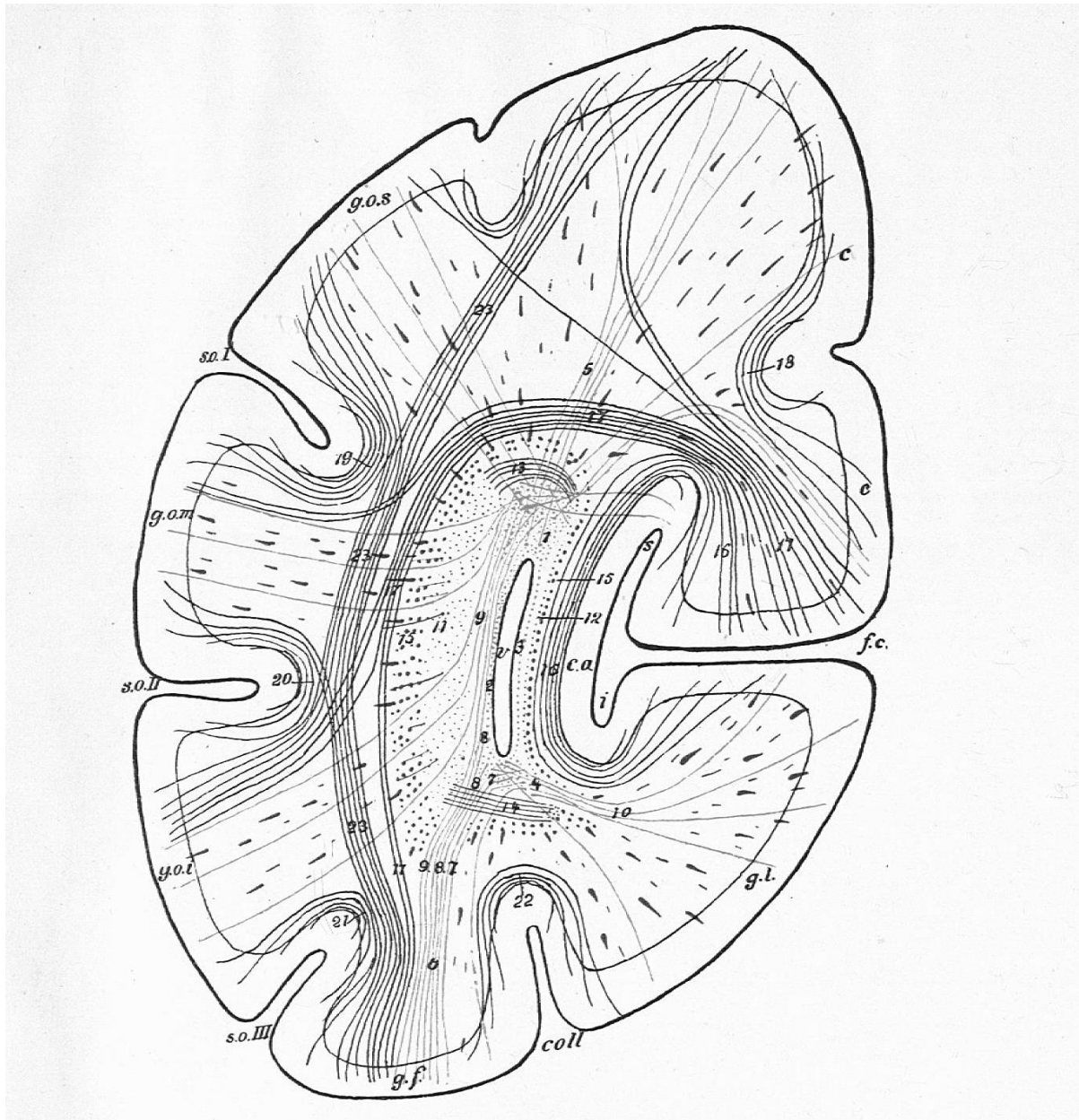


figure 2.1: Schematic representation of the intralobar white matter fibres of the occipital lobe provided by Sachs (1892). Frontal section through a right hemisphere.

v: ventricle (occipital horn); f.c.: fissura calcarina (calcarine fissure); coll.: collateral sulcus; s.o.I: sulcus occipitalis superior, s.o.II: sulcus occipitalis medium; s.o.III: sulcus occipitalis inferior; g.l.: gyrus lingualis; g.f.: gyrus fusiformis; g.o.s.: superior occipital gyrus; g.o.m.: middle occipital gyrus; g.o.s.: superior occipital gyrus; 1-10: forceps; 11-14: stratum sagittale internum; 15: stratum sagittale externum; 16: stratum calcarinum; 17: stratum cunei transversum; 18: stratum proprium cunei; 19: stratum proprium s.o.I; 20: stratum proprium s.o.II; 21: stratum proprium s.o.III; 22: stratum proprium coll.; 23: stratum profundum convexitatis

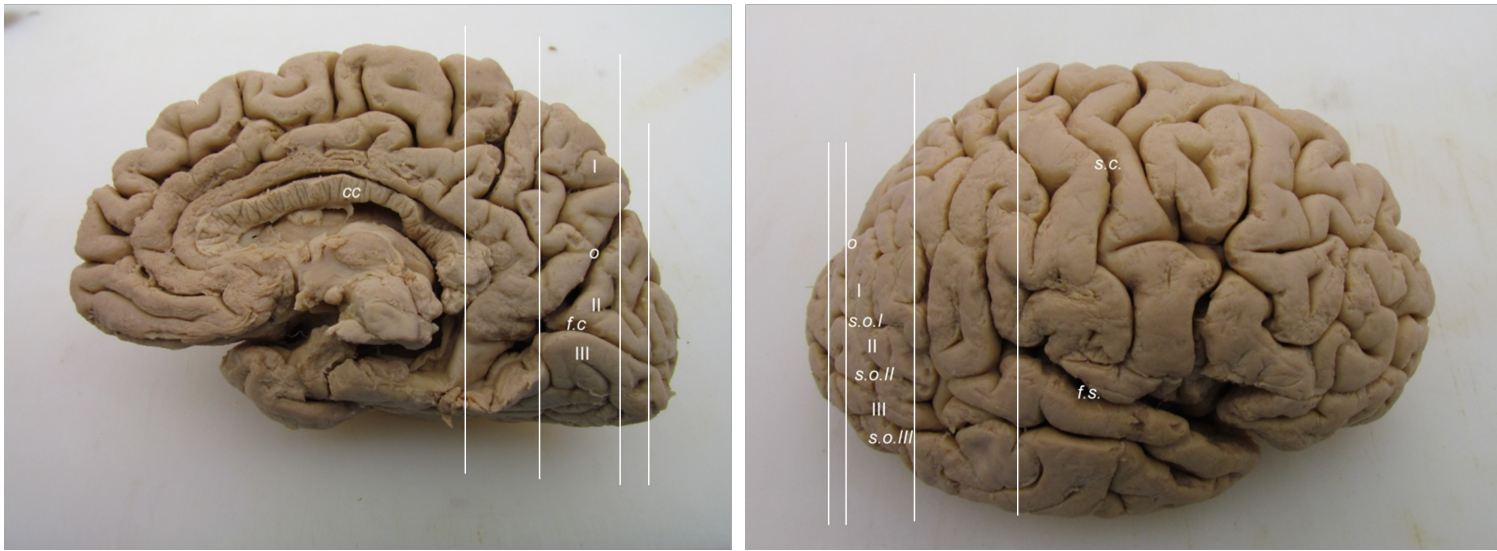


Figure 2.2: A (left panel): medial surface of a right cerebral hemisphere prepared according with Klingler technique. O: occipito-parietal fissure; f.c.: calcarine fissure; cc: corpus callosum; I: precuneus; II: cuneus; III: lingula. B (right panel): lateral surface of a right cerebral hemisphere prepared according with Klingler technique. O: occipito-parietal fissure; f.s.: sylvian fissure; s.o.I: sulcus occipitalis superior; s.o.II: sulcus occipitalis medium; s.o.III: sulcus occipitalis inferior.

Vertical lines correspond to the coronal sections 6, 4, 3 and 2 (from anterior to posterior) as described by Sachs.

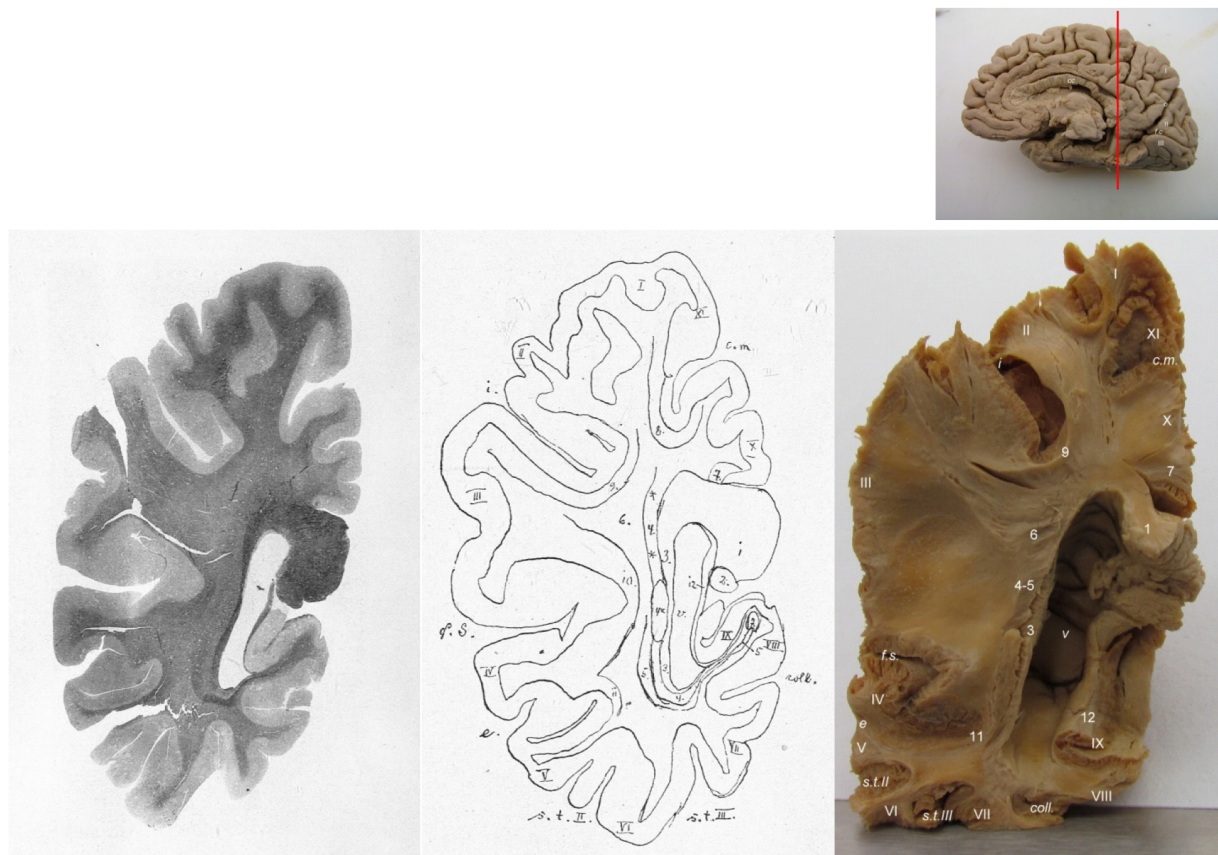


Figure 2.3 coronal section trough the splenium of the corpus callosum (right upper corner). Corresponding histological slice from Sachs atlas(left); schematic diagram of the fibres visualized in the slice (middle); coronal cut of a right-sided hemisphere prepared according to Klingler's method (right) *I*: posterior central gyrus; *II*:superior parietal lobule; *III*: marginal gyrus; *IV*: superior temporal gyrus; *V*: middle temporal gyrus; *VI*: inferior temporal gyrus; *VII*: fusiform gyrus; *VIII*: hyppocampal gyrus; *IX*: Ammon gyrus; *X*: gyrus fornicatus; *XI*: paracentral lobule. *v*: ventricle; *i*: interparietal fissure; *f.s.*: sylvian fissure; *s.t.II*: middle temporal sulcus; *s.t.III*: inferior temporal sulcus; *e*: sulcus parallelis; *coll*: collateral sulcus; *c.m.*: calloso-marginal sulcus; 1: splenium of corpus callosum; 2: inferior portion of foceps; 3: tapetum; 4: stratum sagittale internum; 5: stratum sagittale externum; 6: arcuate fasciculus; 7: cingulum; 8: stratum proprium of the calloso-marginal sulcus; 9: stratum proprium of interparietal sulcus; 10: vertical layer of the convexity; 11: stratum proprium of the sulcus parallelis. 12: column of the fornix

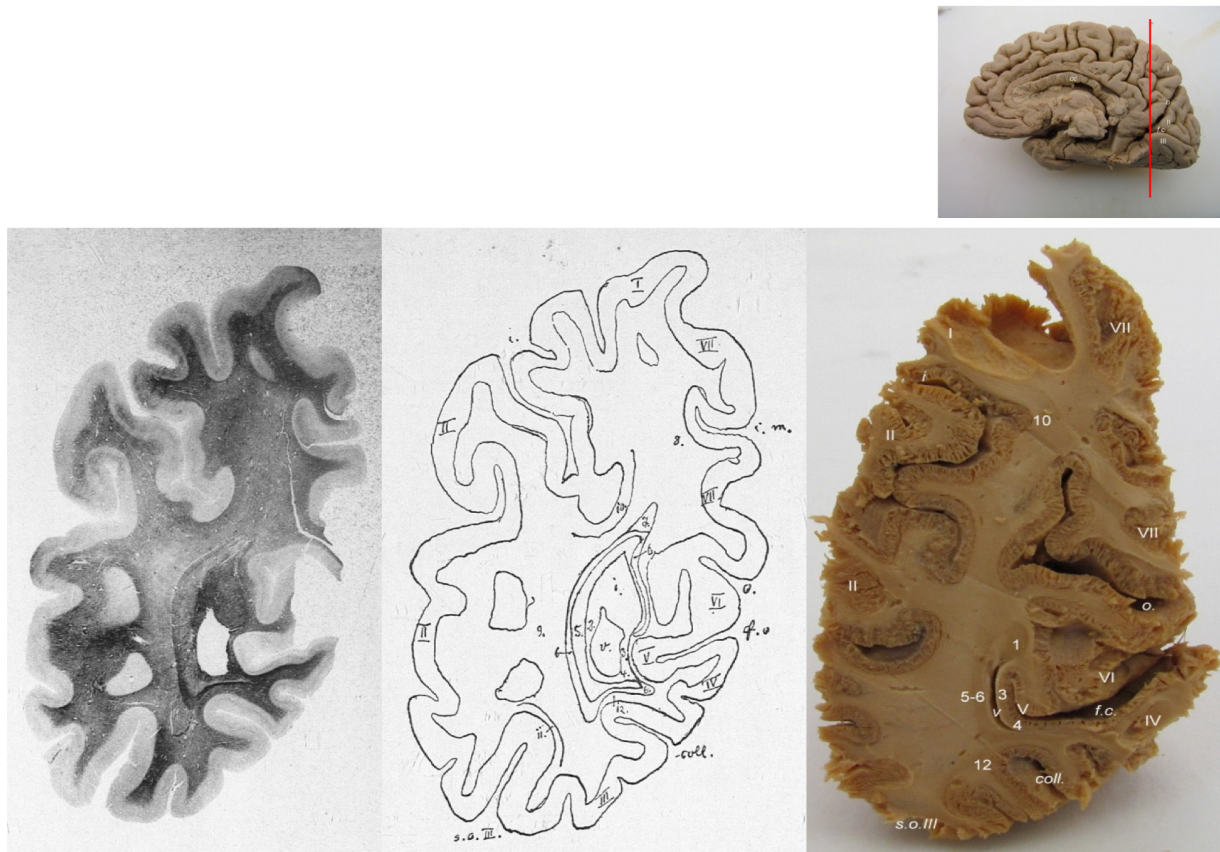


Figure 2.4: Coronal section through the precuneus and anterior edge of calcarine fissure (right upper corner). Corresponding histological slice from Sachs atlas (left); schematic diagram of the fibers visualized in the slice 4 (middle); coronal cut of a right-sided hemisphere prepared according to Klingler's method (right). I: superior parietal lobule; II: angular gyrus; III: fusiform gyrus; IV: lingual gyrus; V: calcar avis; VI: cuneus; VII: precuneus; v: ventricle (occipital horn); i: interparietal fissure; s.o.III: inferior occipital sulcus; c.m.: calloso-marginal fissure; o: occipito-parietal fissure; f.c.: calcarine fissure; coll: collateral sulcus; 1-4: fibres of forceps major; 5: stratum sagittale internum; 6: stratum sagittale externum; 7: stratum transversum cunei (transverse layer of the cuneus); 8: stratum proprium of precuneus; 9: vertical layer of the convexity; 10: stratum proprium of interparietal fissure; 11: stratum proprium of inferior occipital sulcus; 12: stratum proprium collateral sulcus

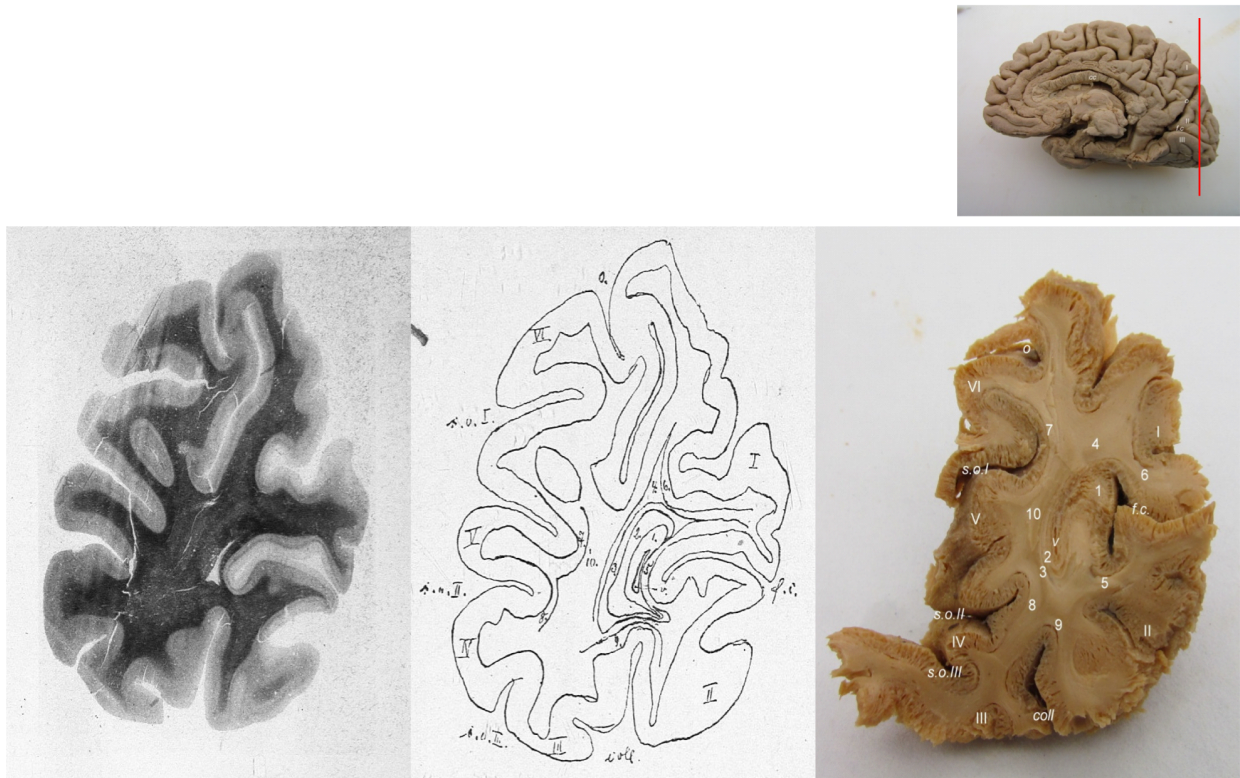


Figure 2.5: Coronal section through the cuneus (right upper corner). Corresponding histological slice from Sachs atlas (left); schematic diagram of the fibers visualized in the slice (middle); coronal cut of a right-sided hemisphere prepared according to Klingler's method (right). I: cuneus; II: lingual gyrus; III: fusiform gyrus; IV: inferior occipital gyrus; V: middle occipital gyrus; VI: superior occipital gyrus; v: ventricle (occipital horn); f.c.: calcarine fissure; coll: collateral sulcus; s.o.I: superior occipital sulcus; s.o.II: middle occipital sulcus; s.o.III: inferior occipital sulcus; o: occipito-parietal sulcus; 1: forceps; 2: stratum sagittale internum; 3: stratum sagittale externum; 4: transverse layer of the cuneus; 5: stratum calcarinum; 6: stratum proprium of the cuneus; 7: stratum proprium of s.o.I; 8: stratum proprium of s.o.II; 9: stratum proprium of collateral sulcus; 10: deep layer of the convexity.

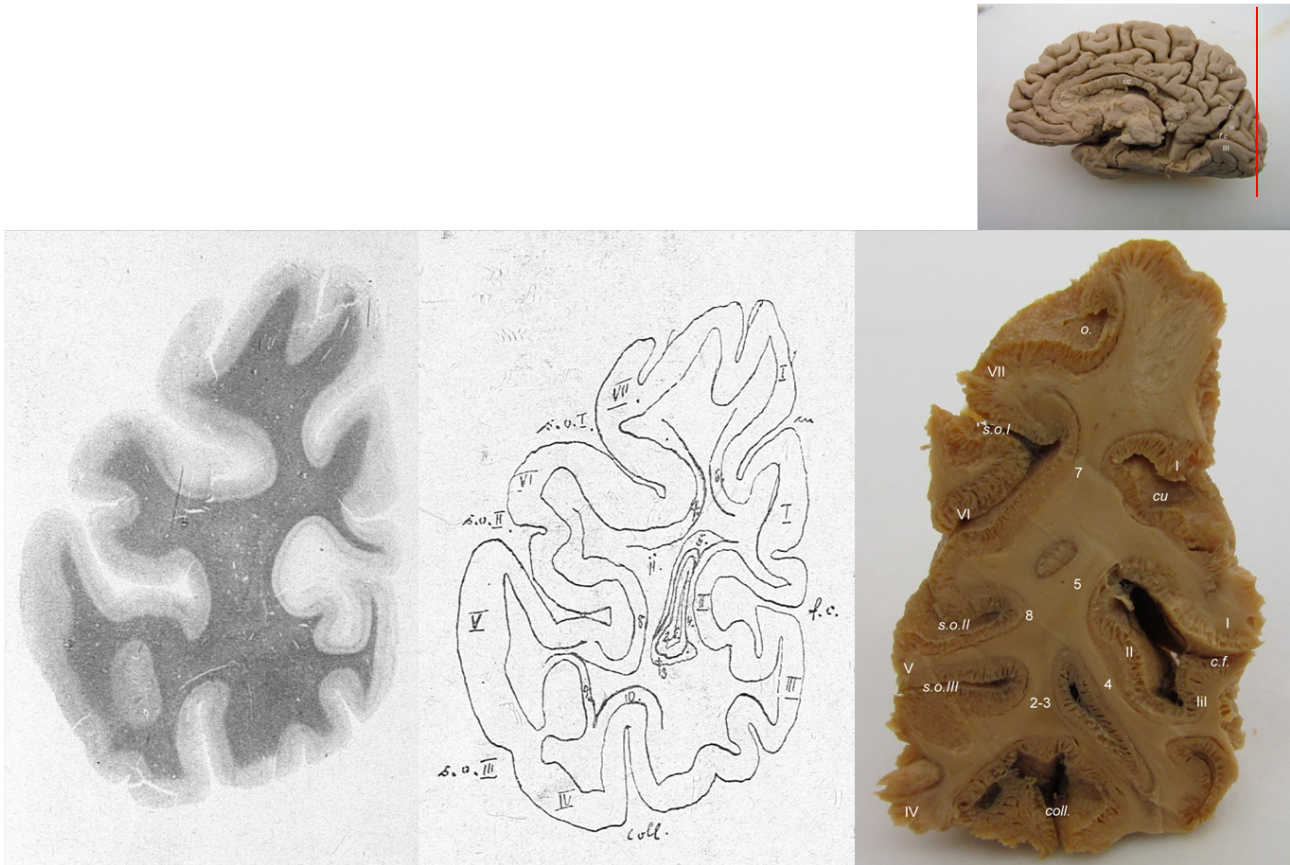


Figure 2.6: Coronal section through the posterior portion of the cuneus (upper right inset). Histological slice corresponding to section 2 of Sachs (left); schematic diagram of the fibers visualized in photo 2 (middle); coronal cut of a right-sided hemisphere prepared according to Klingler's method

I: cuneus; II: calcar avis; III: lingual gyrus; IV: fusiform gyrus; V: inferior occipital gyrus; VI: middle occipital gyrus; VII: superior occipital gyrus; s.o.I: superior occipital sulcus; s.o.II: middle occipital sulcus; s.o.III: inferior occipital sulcus; f.c.: calcarine fissure; coll: collateral sulcus; cu: sulcus of cuneus; 1: forceps; 2: stratum sagittale internum; 3: stratum sagittale externum; 4: stratum calcarinum; 5: transverse layer of the cuneus; 6: stratum proprium of the cuneus; 7: stratum proprium of s.o.I; 8: stratum proprium of s.o.II; 9: stratum proprium of s.o.III; 10: stratum proprium collateral sulcus; 11: deep layer of the convexity

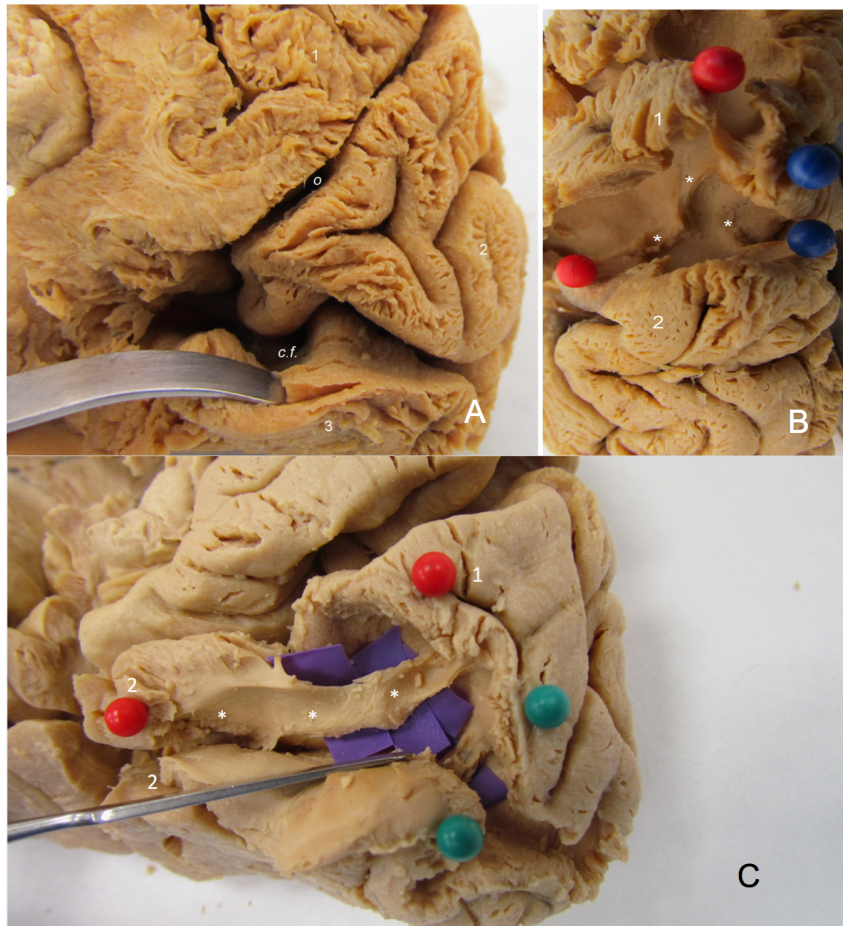


Figure 2.7: dissection of the medial sulci of the occipital lobe (right hemisphere, sagittal view).

- a) medial surface before the dissection. 1: precuneus; 2: cuneus; 3: lingual gyrus. Note the rich pattern of gyri in the calcarine fissure
- b) dissection of the parietoccipital sulcus, showing U fibers (*) laying in the depth of the sulcus and connecting the precuneus (1) with the cuneus (2)
- c) dissection of the calcarine fissure, showing the “slide runner” fascicle (*) running from the cuneus (1) to the antero-superior portion of the lingula (2). U fibers laying in the depth of the sulcus and connecting the upper and lower edges of the calcarine fissure are also demonstrated (green pins). These fibers correspond to the stratum calcarinum of Sachs.

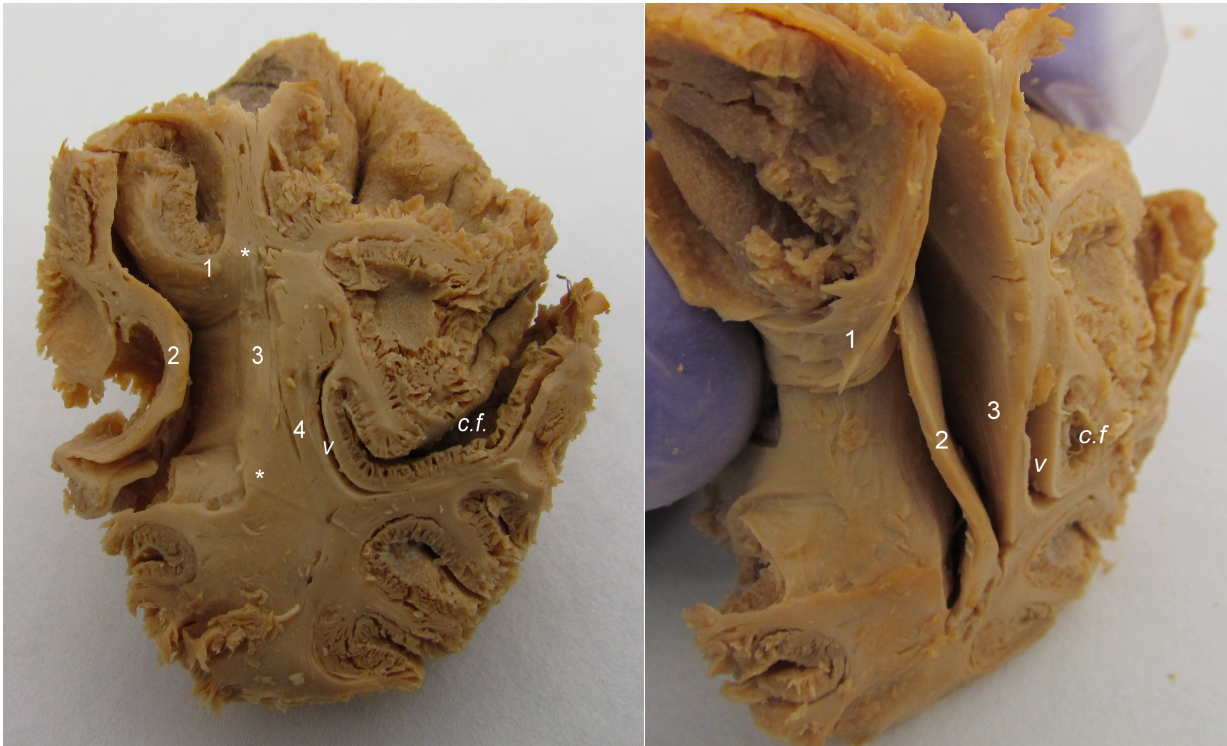


Figure 2.8: lateral to medial dissection of the occipital lobe (right hemisphere, coronal view).

Left: removal of the cortex overlying the sulci at the convexity revealed a system of U fibers connecting neighbouring gyri (1 and 2). These fibers correspond to the *stratum proprium sulci occipitalis* according to Sachs. Deeper to these U fibres, a vertical bundle was identified (3), corresponding to the *stratum profundum convexitatis* described by Sachs. This bundle, lateral to the stratum sagittale (4) appeared to blend with the superficial fibres of the convexity (*). V: ventricle, c.f.: calcarine fissure

Right: dissection carried more medially in the same hemisphere. The vertical *stratum profundum convexitatis* has been partially removed (1) to expose the external (2) and internal (3) layers of the stratum sagittale at the level of the lateral wall of the ventricle (v). Note the longitudinal course of the fibers of the stratum sagittale

3.3 Fourth paper - White matter connections of the Supplementary Motor Area in humans

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The supplementary motor area (SMA) is an eloquent region of the brain that plays an important role in the planning, initiation and learning of complex motor functions. The SMA in the dominant hemisphere is also involved in the initiation of speech. From a neurosurgical perspective, the SMA is important as it is frequently involved by brain tumours and surgery in this area can be followed by the “SMA syndrome”, characterized by contralateral akinesia and, for surgery in the dominant hemisphere, mutism or speech reduction (also referred to as “transcortical motor aphasia”) (Laplane 1977, Duffau 2004). These symptoms tend to resolve over time, but can affect patients for several months and can occasionally be permanent. Knowledge of the connections of the SMA can help to understand the pathogenesis of the SMA syndrome, providing the neurosurgeon with a better understanding of the challenges related to operating in this region, as well as offering patients a more accurate preoperative counselling.

In the present study we adopted for the first time in the literature a combined approach to investigate the SMA connections, using both post-mortem dissections (on 12 hemispheres) and diffusion imaging performed in vivo on healthy volunteers (10 subjects). The study was performed in collaboration with the NatbrainLab of King's College London, which provided the tractography data.

Five main connections were found: 1) U-fibres running in the precentral sulcus, connecting the precentral gyrus and the SMA; 2) U-fibres running in the cingulate sulcus, connecting the SMA with the cingulate gyrus; 3) frontal “aslant” fascicle, directly connecting the SMA with the pars opercularis of the inferior frontal gyrus; 4) medial fibres connecting the SMA with the striatum; 5) SMA callosal fibres. Good concordance was observed between post-mortem dissections and Diffusion tractography.

This study demonstrated that the SMA shows a wide range of white matter connections with motor, language and limbic areas. Features of the SMA syndrome (akinesia and mutism) can be better understood on the basis of these findings.

3.3.1. Results

Each specimen was accurately examined and the major sulci and circonvolutions were identified. The central sulcus, dividing the pre-central (PreCG) and post-central (PoCG) gyri, was the initial landmark to be identified on the lateral convexity. The precentral sulcus, anterior to the PreCG, was used as the posterior border of the SMA. The superior frontal sulcus, separating the superior (SFG) and middle (MFG) frontal gyri, was considered as the infero-lateral border of the SMA (Rostomily 1991). On the medial aspect of the brain, the cingulate sulcus, separating the mesial aspect of the superior frontal gyrus (SFG) and the cingulate gyrus (Cing), represented the infero-medial border of the SMA. The anterior border was considered to be 5 cm in front of the precentral sulcus. The SMA is divided in pre-SMA and SMA-proper by a vertical line passing through the anterior commissure (VCA line). On the medial aspect of each hemisphere the VCA line was traced and the two regions of the SMA were identified (figure 3.1).

Dissection of the lateral aspect of the SMA

Short U fibers

After removal of the cortex, the first fibers to be exposed on the convexity of the hemisphere were short arcuate fibers connecting neighbouring gyri (also known as “U fibers”). In particular, U fibers were encountered in the depth of the precentral sulcus, connecting the posterior part of the SMA-proper with the PreCG. The number and distribution of these fibers appeared to be variable, but a short arcuate fiber was always present in close proximity of the “hand knob” of the primary motor cortex. This is a “knob-like” portion of the PreCG corresponding to the middle knee of the central sulcus and representing the anatomical localisation of the motor hand area (Yousry 1997). Similarly, U fibers were encountered in the depth of the superior frontal sulcus, connecting the lateral aspect of the SMA with the middle frontal gyrus (MFG) (figure 3.2).

Frontal “aslant” tract

Continuing the dissection of the lateral aspect of the frontal lobe a direct fiber tract running from the superior frontal gyrus to the pars opercularis of the inferior frontal gyrus (IFG) (area 46, according to Brodmann’s classification) was demonstrated. After removal of the MFG and partial removal of the IFG, longitudinal fibers of the Superior Longitudinal Fasciculus (SLF) were exposed. Deeper to the SLF, a vertical bundle of fibers, running from the SMA to the pars opercularis of the IFG was observed (figure 3). This fascicle presented an oblique course between the SFG and IFG, and corresponded to the frontal “aslant” fascicle. The cortical termination of this tract in the SFG was at the boundary between the pre-SMA and SMA-proper. (figure 3.3). The tract was clearly demonstrated also with Diffusion tractography, with a predominance of the tract on the left side, as shown by the lateralization index (figure 3.3, table 1).

Table 1	Subj 1	Subj 2	Subj 3	Subj 4	Subj 5	Subj 6	Subj 7	Subj 8	Subj 9	Subj1 0	Mean \pm std
Caudate	0.43 0	0.97 2	-0.1 08	-0.4 03	-0.8 01	2.00 0	1.37 5	0.69 4	1.28 1	1.757	0.7196 \pm 0.9348
Cingulate Gyrus - anterior	0.07 6	0.55 5	-0.2 35	0.82 4	0.56 2	-0.3 51	-0.3 29	0.45 3	-1.0 16	1.344	0.1883 \pm 0.6898
Inferior Frontal Gyrus	0.04 2	0.59 4	0.00 0	0.48 7	0.59 2	-0.0 67	0.34 4	0.40 2	0.07 2	0.333	0.2797 \pm 0.2494
Precentral Gyrus	-0.0 52	-0.3 81	-0.2 54	-0.0 42	-1.1 81	1.03 1	0.67 7	0.22 9	0.60 4	0.551	0.1182 \pm 0.6421

Table 1 - lateralisation index of the tract described.

Dissection of the medial aspect of the SMA

Short U fibers

On the medial aspect of the hemisphere, another set of U fibers was observed, running in the depth of the cingulate sulcus. These fibers connected the SMA to the cingulate gyrus, running perpendicular to the cingulate sulcus. Diffusion tractography confirmed the presence of an extensive connection between the SMA and the anterior portion of the cingulate gyrus (figure 3.4).

Callosal fibers

Dissecting the mesial aspect of the brain, after removal of the cingulate gyrus, vertical fibers of the corpus callosum were exposed. These callosal fibers came from both the pre-SMA and the SMA-proper and converged on the rostrum and anterior part of the body of the corpus callosum (figure 3.5).

Corona radiata and striatal fibers

Continuing the dissection from medial to lateral, after complete removal of the cingulate gyrus and partial removal of the corpus callosum, the caudate nucleus was exposed. At this level, two sets of fibers running vertically from both the SMA-proper and pre-SMA were

identified. The first group to be encountered belonged to the corona radiata and continued inferiorly, with a slight oblique course, into the internal capsule. More laterally, another group of fibers converged directly onto the head and anterior body of the caudate nucleus. These fibers had a more vertical course and were situated more laterally to the callosal and corona radiata fibers. Diffusion tractography also showed a contingent of fibres from the SMA converging onto the head of the caudate nucleus (figure 3.6). These fibres showed a left lateralization (table 1).

3.3.2. Discussion

Methodological aspects

Extensive data about the hodology of the SMA are available in the literature from animal studies (Cipolloni 1999, Hutchins 1988, Jurgens 1984, Luppino 1993). The common method used to investigate the white matter in the primate is the autoradiography technique, where a neuronal tracer is injected in the area of interest and the connections of that particular area are then established with the analysis of post-mortem histology. This method can be considered as the “gold standard” for the study of white matter connections, and a complete reconstruction of the fibers of the monkey brain has been provided (Schmahmann and Pandya 2009). In humans, two techniques are available to investigate the white matter anatomy: post-mortem dissection and Diffusion tractography.

Dissection of cadaveric specimens has been used for centuries to study the anatomy of the brain, including the tracts of white matter. In recent years, the technique originally

described by Klingler (Klingler 1935) has been revitalised in the neurosurgical community (Ture 2000). The crucial step in the “Klingler method” is the freezing process: the ice that forms between the fibers separates the fibre bundles, thus facilitating their dissection (Ludwig and Klingler 1956). A modification of this technique has been recently suggested, with the aim of sparing the cortex during the dissection (so called “cortex-sparing” dissection) (Martino 2011b). The advantage is maintaining the three dimensional relationship between the fiber tracts and their cortical terminations, thus avoiding the loss of fundamental landmarks during the dissection.

Diffusor Tensor Imaging is a relatively new MRI technique, based on the principle that the diffusion of water molecules in white matter tracts is anisotropic. Tractography can reconstruct the direction of fiber tracts, with the unique advantage of investigating the white matter anatomy in vivo (Jones 1999, LeBihan 2001, Catani 2008b). One of the limitations of DTI is that it is unable to accurately demonstrate the cortical termination of a fiber tract, as this can only be inferred from the location and orientation of the average tract end points (Catani 2003).

To the best of our knowledge, the present study is the first in the literature dedicated to investigate the white matter connections of the SMA using both post-mortem dissections (according to the traditional Klingler technique and the “cortex-sparing” method) and Diffusion tractography. A good concordance was found between the two methods. All the connections described were identified with both techniques, with similar trajectories and directions of the fiber tracts observed. Post-mortem dissection (particularly the “cortex-sparing”) was able to clearly identify the cortical termination of the bundles, showing the relationship of the fibers to other white matter tracts and to adjacent cortical landmarks.

SMA and movement

Direct stimulation of the SMA induces a complex pattern of movements, involving different muscles groups. Synergistic movements involving the head, the upper and lower limb (turning the head to look at the hand, grasping movements, etc..) have been described (Penfield and Rasmussen 1950, Chauvel 1996, Hanakawa 2001, Fontaine 2002, Chassagnon 2008). It is acknowledged that the SMA plays an important role in planning, initiation and execution of movements. In the primate, an SMA-proper and a pre-SMA have been differentiated on the basis of cytoarchitectonic and functional aspects (Rizzolatti 1996). Also in humans, in addition to the classic SMA (SMA-proper), a more rostral area, called “pre-SMA” has been described. The role of these two regions appear to be different with regard to motor function, with the pre-SMA being involved in higher motor control, such as planning and preparation of movement, while the SMA-proper is more directly related to the execution of movement (Orgogozo 1979, Rao 1993, Shibasaki 1993, VanOostende 1993, Ikeda 1999). The connections of the two regions can provide an anatomical basis for these functional aspects. It is known from the literature (Picard 1996, Rizzolatti 1996) that the SMA-proper sends fibers through the corona radiata and the internal capsule to join the cortico-spinal tract, while the pre-SMA has no direct connection with the spinal cord. Thanks to this cortico-spinal projection, the SMA-proper is thought to play a role in the direct execution of movement. In addition, our study has demonstrated the presence of short U fibers in the depth of the precentral sulcus, directly connecting the SMA-proper with the primary motor cortex (M1). Of interest, a direct link between the SMA-proper and the M1 appears to be always present at the level of the hand region. It has been hypothesized that the convergence of fibers at this level can play a role in the control of complex hand movements, such as grasping and reaching (Catani 2012). Finally, both the SMA-proper and the pre-SMA have direct connection with the striatum,

with fibers directed to the head and body of the caudate nucleus. The presence of striatal connections has been previously demonstrated in a DTI study by Lehericy et al. (Lehericy 2004), with the SMA-proper sending fibers to posterior regions of the striatum (sensorimotor compartment), while the pre-SMA sends connections to more anterior portions of the striatum, including the associative compartment. The results of our study are in accordance with these findings. This cortico-striatal connection is part of a wider network that, through the thalamus, reverberates back to the cortex (Alexander & Crutcher 1990, Parent & Hazrati 1995). This cortico-basal ganglia-thalamo-cortical network is implicated in different aspects of motor control, including initiation, sequencing and modulation of voluntary movements (Alexander & Crutcher 1990). The result of our Diffusion tractography study suggest a left lateralization of these fibres in right-handed individuals.

SMA and language

Direct stimulation of the dominant SMA has been related to vocalization and/or arrest of speech (Penfield and Rasmussen 1950, Chassagnon 2008). Functional MRI studies have provided useful insights in the role of the SMA in language. The SMA appeared to be activated during different language tasks, such as repetition and silent verbal fluency, with the role of facilitating the initiation of speech (Benson 1999, Lehericy 2000, Crosson 2001). In the present study we demonstrate the presence of a direct connection between the SMA and the pars opercularis of the IFG, corresponding to Broca's area in the dominant hemisphere. This tract has been called the "frontal aslant tract" (FAT) due to its oblique course (Catani 2012). Our results are in accordance with the previous description of the bundle provided by DTI studies (Ford 2010, Klein 2007, Oishi 2008). More recently,

Kinoshita et al. have described a similar bundle connecting the medial frontal cortex and the IFG (Kinoshita 2012). The Klingler dissection performed by the authors suggests that the fibers of the FAT are merging with the SLF. In our study, the FAT appears to be deeper than the SLF, which had to be cut in order to expose the underlying FAT. The SMA is thought to facilitate speech initiation due to the direct connection to the pars opercularis of the IFG provided by the FAT (Catani, 2012). In a study of the somatotopy of the SMA, Fontaine et al. found that the language responses in the SMA were clustered at the border between the pre-SMA and SMA-proper (Fontaine 2002). Interestingly, the border between pre-SMA and SMA-proper represents the cortical termination of the FAT, as demonstrated in the present study by both post-mortem dissections and Diffusion tractography. Finally, the left lateralization of the FAT seen in our Diffusion tractography is also consistent with a possible role in language played by this tract.

In the non-dominant hemisphere it has been suggested that the SMA may mediate voluntary control of face, tongue, and pharynx movement through the FAT connecting the SMA and frontal operculum. If the FAT is damaged, the information coming from the SMA cannot reach the oropharyngeal motor cortex, causing a selective volitional palsy as described in a case of Foix-Chavanie-Marie syndrome after removal of a right-sided fronto-insular low grade glioma (Martino 2012).

SMA and limbic system

Studies from the primate have demonstrated extensive connections between the SMA and the limbic system, particularly through fibers directed to the cingulate gyrus (Morecraft and VanHoesen 1992, Luppino 1993). Our study confirms the presence of such connections in humans, where several U fibers were visualised (both on dissection and Diffusion

tractography), running between the SMA and the cingulate. This direct link between motor and limbic areas is thought to play a role in the motor processing of negative emotional stimuli (LeDoux 1987, Hyman 1998, Northoff 2000). Oliveri and colleagues showed that conditioning transcranial magnetic stimulation of the SMA significantly increased the Evoked-Motor Potential (MEP) recorded from the first interosseous muscle during visual-emotional triggered movements (the subjects had to perform movements in response to images with a negative emotional content). The SMA in humans may therefore interface the limbic and the motor systems in the transformation of emotional experiences into motor actions (Oliveri 2003). The fibers connecting the SMA and the cingulate gyrus are one anatomical substrate for this interaction.

White matter connections and the SMA syndrome

Surgery in the SMA can be followed by the “SMA syndrome”, originally described by Laplane in 1977 (Laplane 1977). Reporting the results of the ablation of the SMA in three patients, Laplane described the clinical course after surgery as being characterised by global akinesia, more prominent contralaterally, with an arrest of speech. This was followed by a phase of recovery, when contralateral reduction of movement and severe reduction of speech were still present. This was followed by a complete recovery, when only a “disturbance of the alternating movements of the hands” (Laplane 1977) was present. Several surgical series related to tumours infiltrating the SMA have confirmed the original observations of Laplane (Rostomily 1991, Zentner 1996, Fontaine 2002, Russell 2007, Krainik 2001, Krainik 2003), usually with more profound and marked symptoms following more extensive resection of the SMA, as it is the case with WHO grade II gliomas (Russell 2007). The outcome is usually favourable, with a complete recovery observed at

6-12 months after surgery. Minor symptoms, however, can be permanent (underutilization of contralateral upper limb, mild word finding difficulties, etc) (Krainik 2001, Krainik 2003).

The pathogenesis of the SMA syndrome is not fully understood. Our findings suggest that surgical ablation of the posterior SMA (SMA-proper) is more likely to induce a motor deficit, as this region is strongly connected with motor areas, through fibers that directly join the cortico-spinal tract and U-fibers directed to the motor cortex. Specific motor symptoms, like deficit in grasping/reaching can indeed be explained on the basis of the connection between SMA-proper and the hand region. The wide range of projections from the SMA to the striatum can provide the anatomical basis to explain the reduction in spontaneous movement, which is the hallmark of the SMA syndrome. Akinesia, a cardinal symptom of parkinsonian disorders, can be the result of a damage to the cortico-striatal network, in which the SMA plays an important role in facilitating and sequencing voluntary movements. Finally, mutism and language reduction (transcortical motor aphasia), can be explained on the basis of the connection of the SMA with the Broca's area provided by the FAT. Indeed, some features of the classic "Broca" aphasia are similar to the mutism observed in the SMA syndrome. The role of the FAT in language is also suggested by the fact that, as demonstrated by our tractography results, the tract is more prominent in the left (dominant) hemisphere.

Knowledge of the anatomical connections of the SMA is relevant to the clinical implications of surgery in the SMA region. The degree of cortical and subcortical extension of SMA tumours (as assessed by preoperative MRI and DTI) can help to predict the postoperative course of patients. This is important to offer patients a more informed counselling on the postoperative course of the SMA syndrome, as well as to plan in advance an individualised rehabilitative intervention.

3.3.3. Conclusions

The SMA is at the centre of a rich network of white matter connections with motor, language and limbic areas. The clinical aspects of the SMA syndrome, including akinesia and mutism, can be better understood on the basis of these anatomical findings.

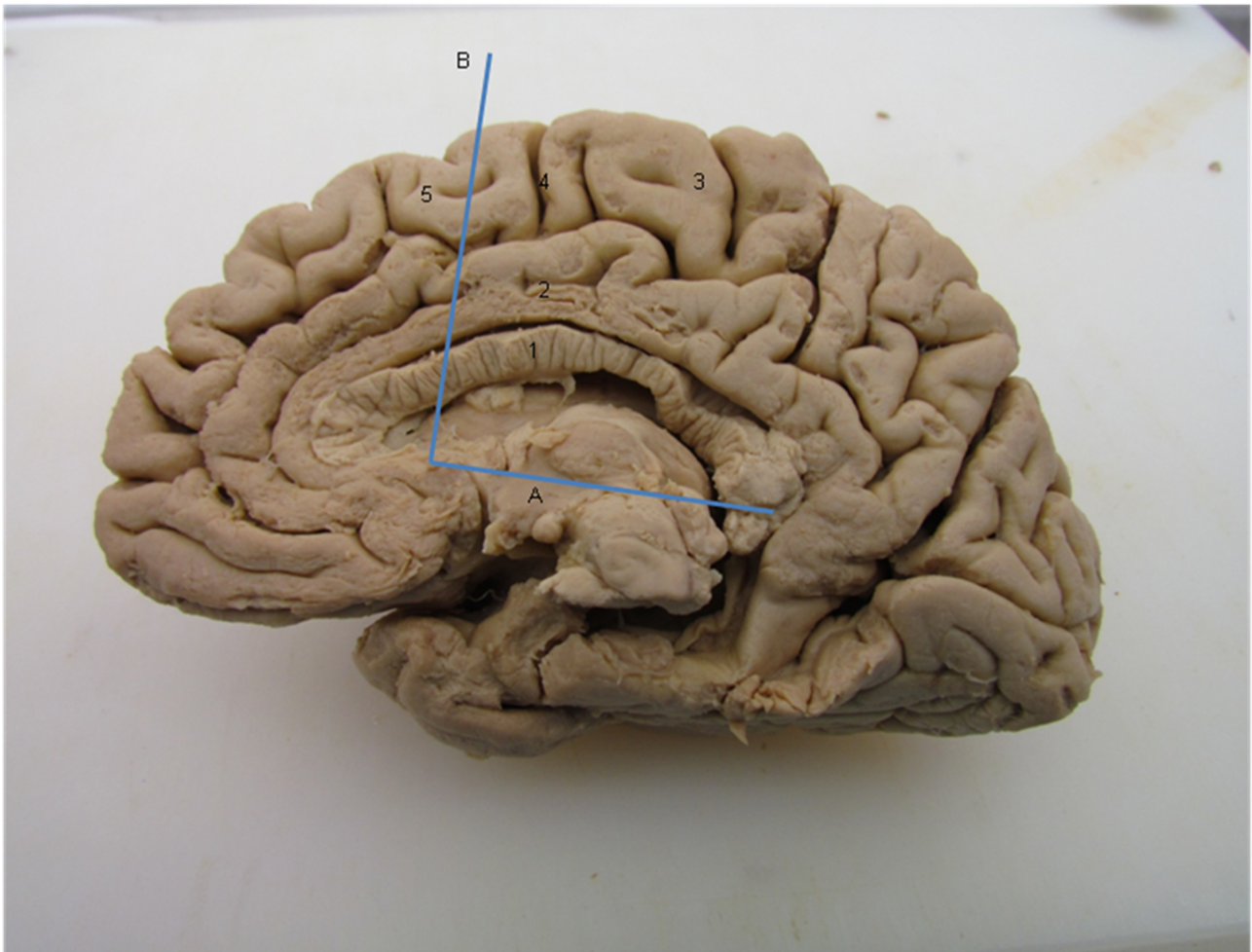


Figure 3.1: medial aspect of a right hemisphere. The AC-PC line is demonstrated (A). The VCA line (B) – perpendicular to the AC-PC line at the level of the anterior commissure - divides the pre-SMA anteriorly (5) from the SMA-proper (4). The corpus callosum (1), cingulate gyrus (2) and central lobule (3) are also indicated.

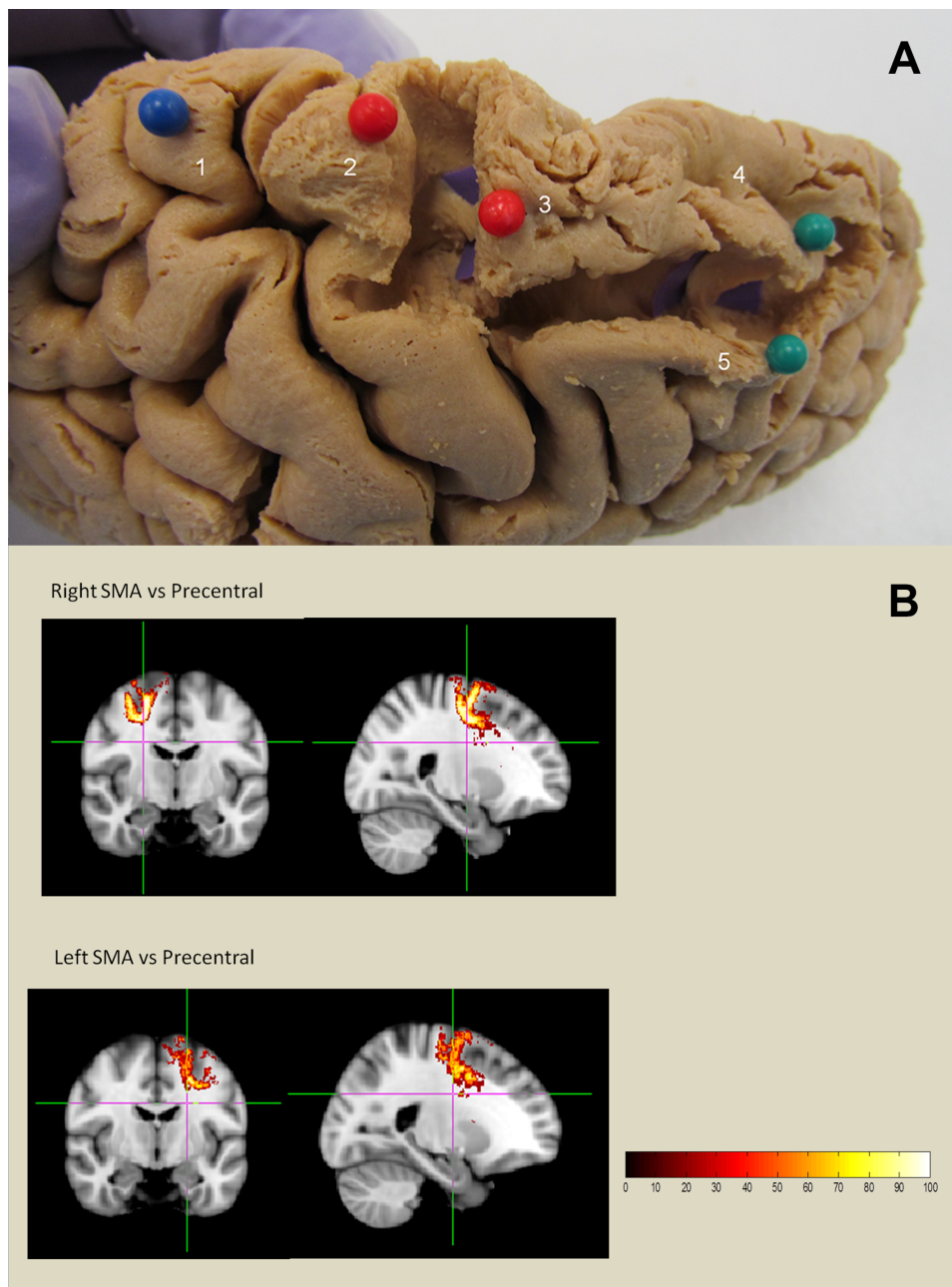


Figure 3.2a: lateral dissection of the SMA (right hemisphere). U-fibres are demonstrated in the depth of the precentral sulcus, connecting the PreCG and SMA-proper (red pins). U-fibres are also present in the superior frontal sulcus, connecting the SMA with the MFG (green pins). 1: PoCG; 2: PreCG; 3:SMA-proper; 4:SFG; 5:MFG

Figure 3.2b: Diffusion tractography demonstrating the right and left connections of the SMA with thePreCG. Note the U-shaped fibres linking the two gyri.

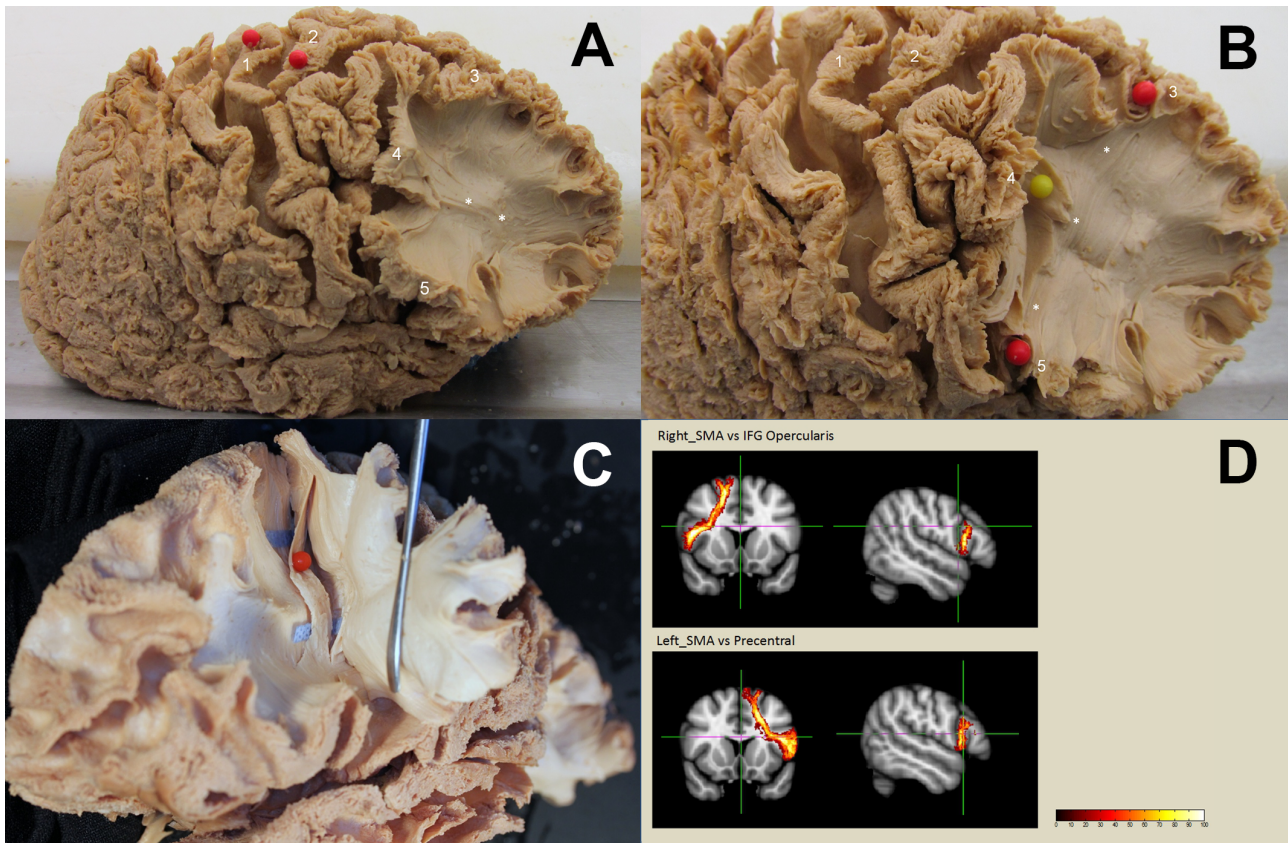


Figure 3.3a: dissection of lateral aspect of the frontal lobe (right hemisphere): exposure of longitudinally-directed fibres of the SLF (asterisk) after removal of the MFG. 1: PoCG; 2: PreCG; 3:SMA; 4:MFG (remnant portion); 5:IFG

Figure 3.3b: same specimen. The SLF has been divided (yellow pin), exposing the vertically oriented fibres of the FAT, connecting the SMA with the pars opercularis of the IFG (red pins). 1: PoCG; 2: PreCG; 3:SMA; 4:MFG (remnant portion); 5:IFG

Figure 3.3c: “cortex sparing” dissection of the left hemisphere. Exposure of the FAT (red pin). The MFG has been maintained and retracted by a blunt dissector. Note the cortical termination of the aslant tract at the level of the SMA and of the opercular portion of the IFG.

Figure 3.3d: Diffusion tractography demonstrating the right and left FAT, connecting the SMA and the IFG.

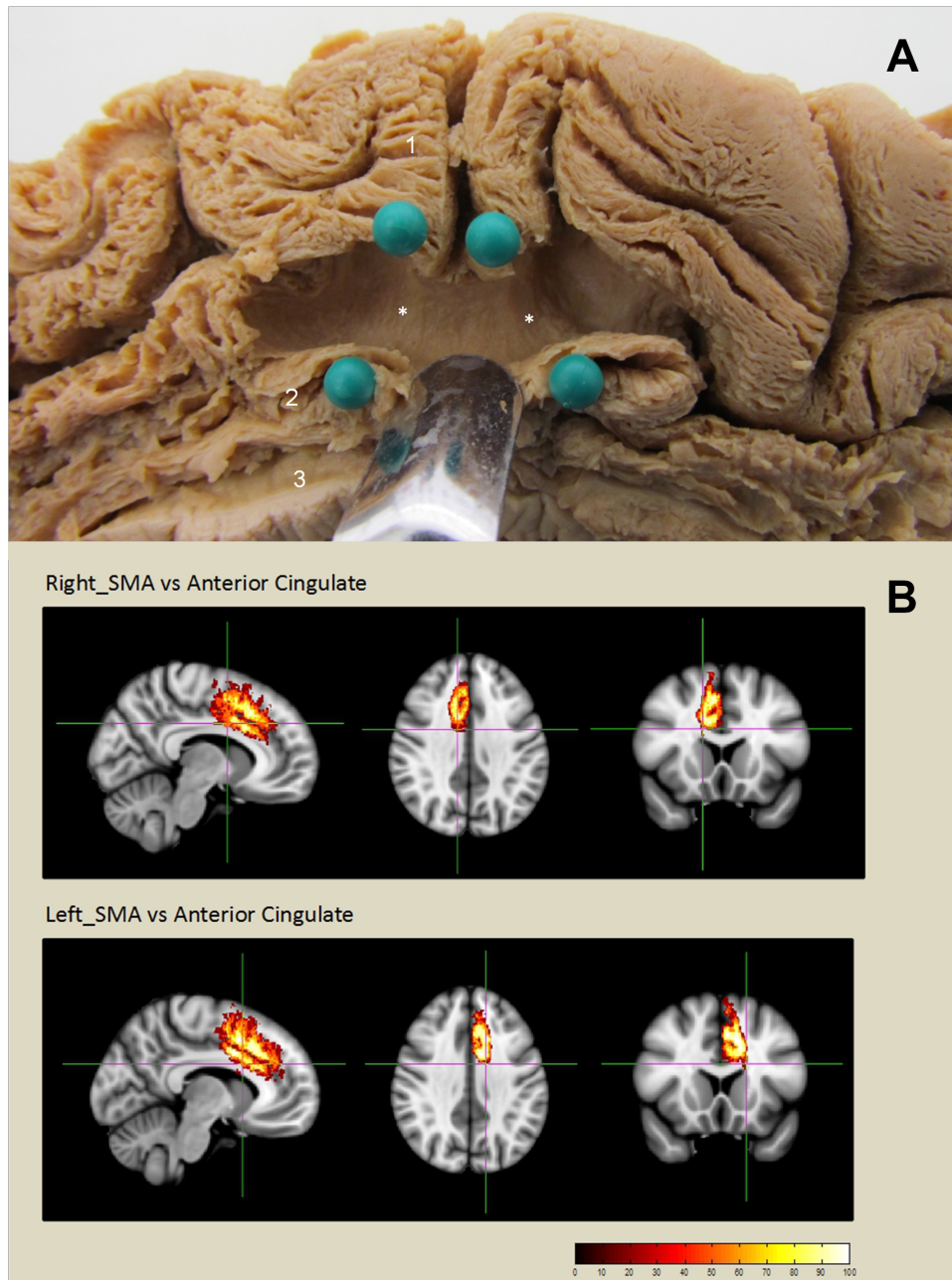


Figure 3.4a: Dissection of mesial aspect of the SMA (right hemisphere). U-fibers running in the cingulate sulcus, connecting the SMA with the cingulate gyrus (asterisks). The green pins show the cortical terminations of these U-fibers. 1:SMA; 2:cyngulate gyrus; 3:corpus callosum.

Figure 3.4b: Diffusion tractography demonstrating the connections between the SMA and the cingulate gyrus. Note the extensive connections with U-fibers to the anterior portion of the cingulate gyrus.

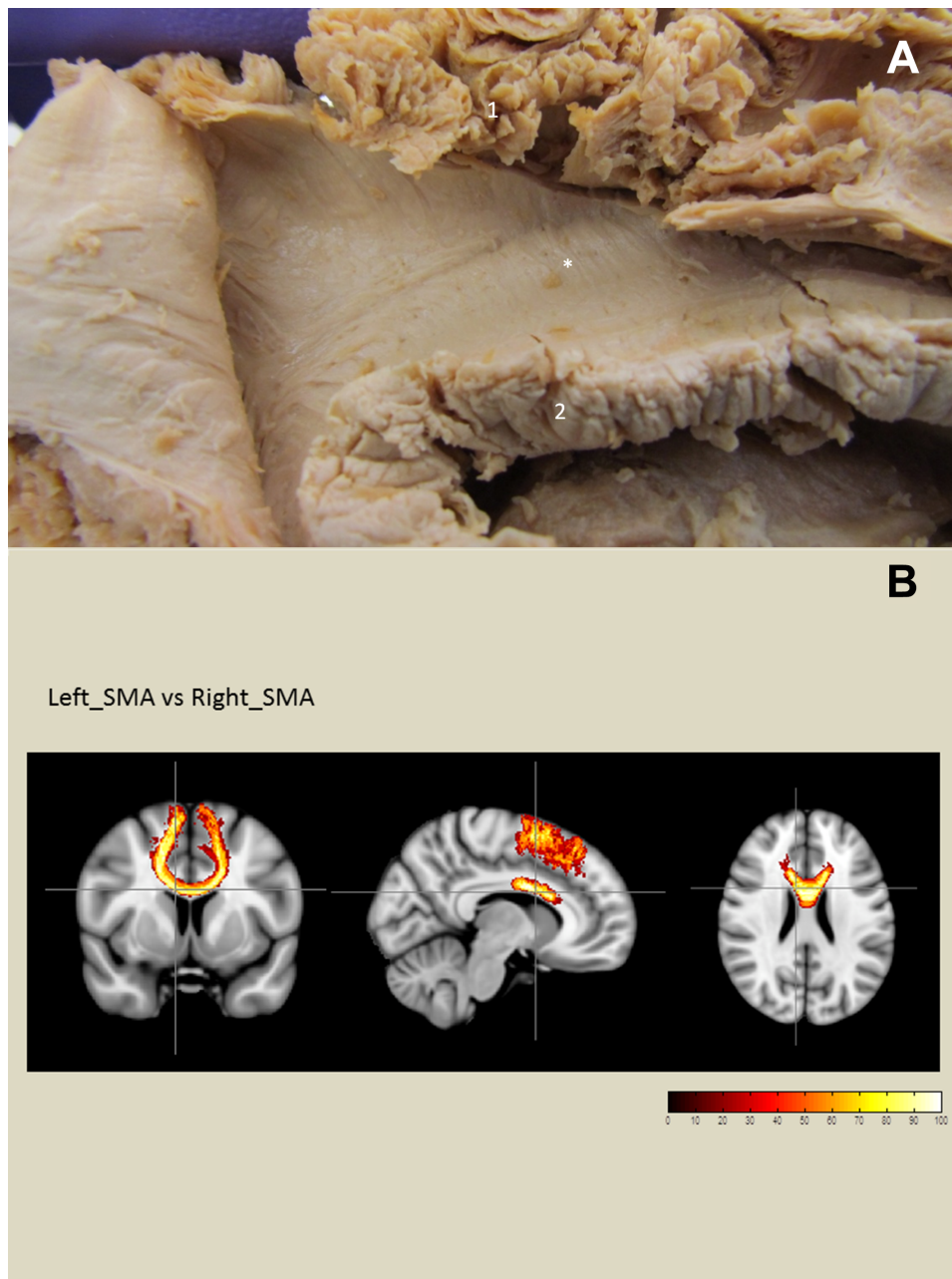


Figure 3.5a: Dissection of mesial aspect of the SMA (right hemisphere). Callosal fibres to the SMA observed after removal of the cingulate gyrus (asterisk). 1: SMA; 2: corpus callosum

Figure 3.5b: Diffusion tractography demonstrating the callosal fibres connecting the left and right SMA.

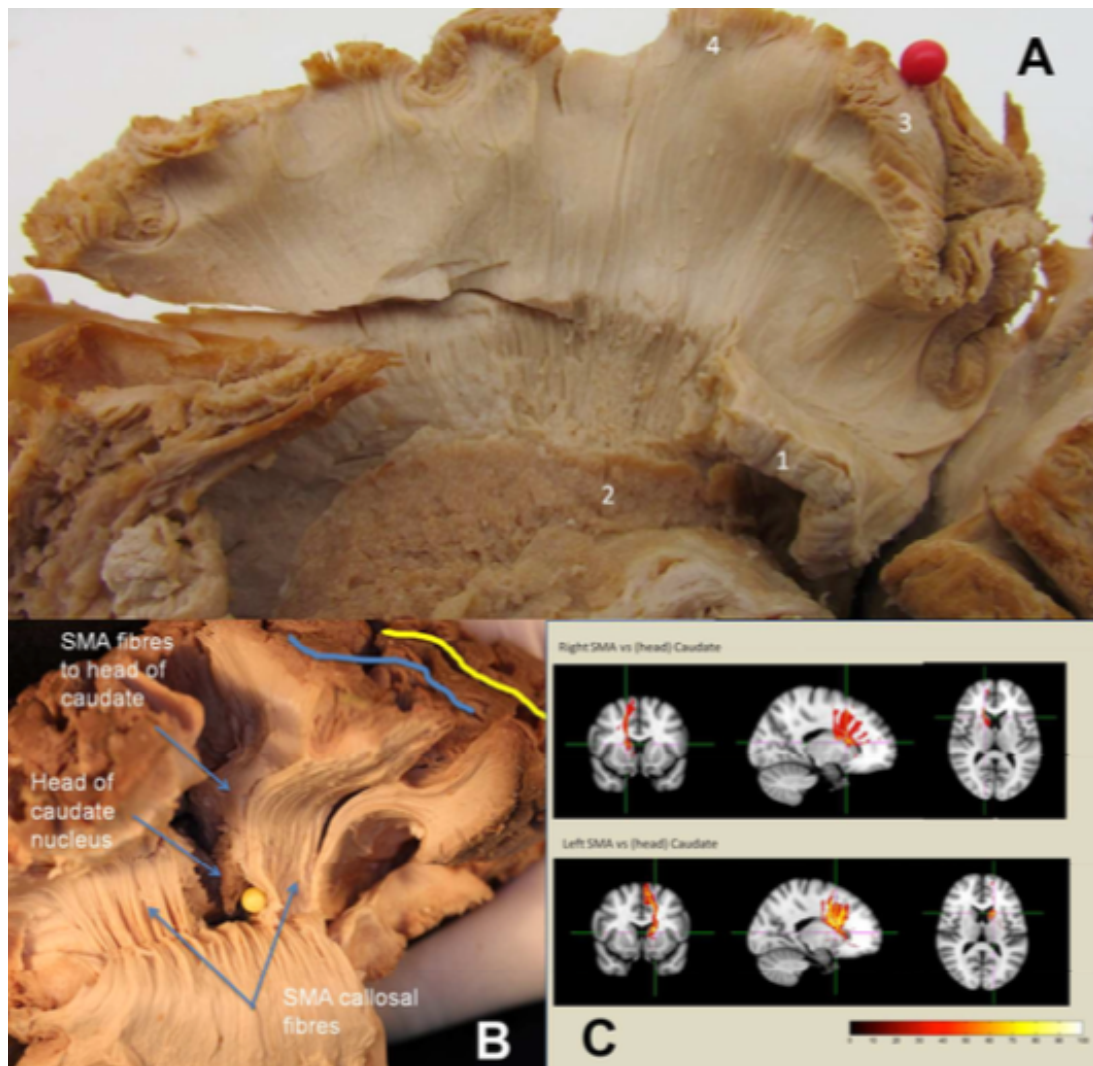


Figure 3.6a: Dissection of mesial aspect of the SMA (right hemisphere). Vertical fibres running from the SMA and converging on the caudate nucleus, seen after complete removal of the cingulate gyrus and of the corpus callosum. Red pin represents the PreCG. 1:corpus callosum (remnant portion); 2:caudate nucleus; 3:PreCG; 4:SMA

Figure 3.6b: “cortex sparing dissection” of a left hemisphere. The relationship between the SMA fibres to the head of caudate nucleus and the SMA callosal fibres is demonstrated. Yellow pin: ventricle. Blue line: precentral sulcus. Yellow line: central sulcus

Figure 3.6c: Diffusion tractography demonstrating the connections between the SMA and the caudate nucleus (right and left hemisphere). Note the convergence of the fibres onto the head of the caudate.

3.4. Fifth paper: Anatomical connections of the Subgenual Cingulate Region

Francesco Vergani, Juan Martino, Johannes Attems, Christopher Morris, Keyoumars Ashkan, Flavio Dell'Acqua

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The idea of investigating the white matter connections of the Subgenual Cingulate Gyrus (SCG) came from the interest that this important “hub” has received in recent years as a target for neuromodulation in psychiatric disorder. The SCG is the portion of cingulate cortex located beneath the genu of the corpus callosum, corresponding to Brodmann’s area 25. Deep Brain Stimulation (DBS) of the white matter underlying the SCG has been proposed for treatment-resistant depression with promising results and more recently, DBS of the SCG has also been proposed for treatment-refractory anorexia nervosa. It is thought that DBS can ameliorate the symptoms of depression and anorexia nervosa by modulating the network of which SCG is a key component. A detailed knowledge of the connections of the SCG can therefore help to elucidate the mechanism of DBS of the SCG. At the same time, a better understanding of the anatomy of the white matter underlying the SCG can assist the neurosurgeon in refining the positioning of the electrode within the SCG target.

We were again the first to use a combined approach (using both post-mortem dissections on 10 hemispheres and tractography on 20 healthy subjects) to investigate the SCG connections. Tractography was obtained in collaboration with the NatbrainLab of King’s College London.

Four main connections were found: 1) fibres of the cingulum, originating at the level of the SCG and terminating at the medial aspect of the temporal lobe (parahippocampal gyrus);

2) fibres running towards the base of the frontal lobe, connecting the SCG with fronto-polar areas, 3) fibres running more medially, converging onto the ventral striatum (nucleus accumbens), 4) fibres of the uncinate fasciculus, connecting the orbito-frontal with the anterior temporal region.

This study shows that the white matter fibres deep to the SCG are at the centre of a large network, connecting prefrontal, limbic and mesotemporal regions. Two large associative bundles related to the limbic system, the cingulum and the uncinate fasciculus, are part of the SCG network. Short intralobar fibres connect the SCG to the fronto-polar region and the fronto-polar region to the accumbens. Comprehensive knowledge of the anatomy of these fibres can help explain the clinical effect of neuromodulation in neuropsychiatric disorders.

3.4.1. Results

The SCG was identified on the medial aspect of each hemisphere. The cingulate gyrus was in direct continuation with the SCG, following the contour of the corpus callosum. The sulcus of the corpus callosum divided the cingulate gyrus (superiorly) and the corpus callosum (inferiorly). The cingulate sulcus divided the cingulate gyrus (inferiorly) from the mesial aspect of the superior frontal gyrus (superiorly). At its inferior edge, the cingulate sulcus divided the SCG (posteriorly) from the paraolfactory gyrus anteriorly. Posteriorly, the SCG was bordered by the rostrum of the corpus callosum and the anterior commissure (figure 4.1).

Cingulum

The first fibres encountered dissecting the SCG cortex were fibres belonging to the cingulum. Completing the removal of the cingulate gyrus, the cingulum was thus progressively exposed. It appeared as a C-shaped tract running deep to the cingulate gyrus and developing around the corpus callosum. From its most anterior aspect, at the level of the SCG, this tract runs longitudinally passing anteriorly to the genu and superiorly to the body of the corpus callosum. Posteriorly to the splenium, at the level of the isthmus of the cingulate gyrus, the tract narrows taking an anterior course and extending into the mesial aspect of the temporal lobe, where it terminates at the anterior portion of the parahippocampal gyrus, adjacent to the hippocampus. Distinct groups of superficial vertical fibres were observed running between the cingulum and adjacent gyri. Anteriorly, small vertical fibres to the superior frontal gyrus and to the paracentral lobule were identified. More posteriorly, a larger and more easily identifiable group of vertical fibres was directed between the cingulate and the precunes (figure 4.2 and 4.5).

Fronto-polar connections

A group of fibres was observed running from the SCG anteriorly, towards the basal and mesial aspect of the frontal pole. Inferiorly, after removing the cortex of the paraolfactory gyrus, a short longitudinal tract was exposed. This tract runs between the SCG and the medial aspect of the frontal pole (corresponding to Brodmann's areas 11 and 12). More superiorly, another short longitudinal tract was observed between the SCG and the inferior portion of the medial frontal gyrus (medial part of the superior frontal gyrus), corresponding to Brodmann area 32. These prefrontal connections appeared to converge posteriorly

towards the SCG, almost blending with the posterior portion of the cingulum (see red pin in figure 4.2 and 4.5).

Uncinate fasciculus

Dissection of the inferior portion of the medial frontal lobe, with removal of the paraolfactory gyrus and partial removal of the rectus gyrus, exposed fibres running posteriorly and inferiorly with respect to the SCG. These fibres have a hook-shaped course, curving anteriorly to the ventral portion of the striatum to form the mesial part of the temporal stem. The fibres continue their course until reaching the most anterior aspect of the mesial temporal pole, adjacent to the amygdala. These fibers correspond to the mesial portion of the uncinate fasciculus, which runs from the SCG and gyrus rectus to the mesial temporal pole (figure 4.3 and 4.5).

Callosal and striatal fibres

Continuing the dissection in a medial direction, after complete removal of the cingulum and prefrontal fibres, the deeper fibres of the corpus callosum were exposed. These fibres have a fan-like appearance and run anteriorly and laterally, contributing to the forceps minor. At the most inferior edge of the dissection, deep to the rectus gyrus, a small bundle was observed running from the frontal pole in a posterior direction, converging onto the ventral portion of the striatum (at the site of the nucleus accumbens). This bundle corresponds to the previously described accumbo-frontal fascicle (Rigoard, 2011). It runs through the deep white matter of the SCG, superiorly to medial part of the uncinate fasciculus and terminates at the level of the ventral striatum (figure 4.4 and 4.5).

3.4.2. Discussion

The connectivity of SCG

In the present paper, we present a comprehensive description of the connections of the SCG. This area appears to be at the centre of a rich network of fibres, connecting the SCG to the cingulate, hippocampus, amygdala, orbitofrontal cortex and ventral striatum. To the best of our knowledge, this is the first time that these connections have been investigated using a combined approach, employing both post mortem dissections and advance diffusion imaging. Previous studies employed probabilistic tractography to investigate the connections of the SCG in humans (Johansen-Berg 2008, Gutman 2009). The results of these studies demonstrated an overall similar pattern of connections, with projections to nucleus accumbens, amygdala, hypothalamus, and orbitofrontal cortex. In our study, connections to the amygdala and anterior part of the temporal lobe appeared to be mediated via the mesial portion of the uncinate fasciculus, while connections to the hippocampus are mediated via the cingulum. Similarly, we identified short intralobar frontal fibres directed to the fronto-polar cortex. In our study, we did not observe a direct connection to the hypothalamus, whereas fine fibres were seen converging onto the ventral portion of the striatum/nucleus accumbens. It has to be noted that probabilistic tractography has some methodological limitations (Johansen-Berg 2008). ROI sizes, along with the geometrical property (size and dimension) of the bundles studied can affect the final results of tractography, introducing false positive or false negative results. Our approach has the merit of combining the results of a traditional anatomical technique – the Klinger dissection - with advance diffusion tractography. All the SCG connections described in the present paper were identified with both techniques, with similar trajectories and directions of the fibre tracts observed.

The first, large connection to the SCG identified was the cingulum. This fascicle has been extensively studied in the primate and in humans. These studies demonstrated that the cingulum contains fibers from the cingulate cortex to isocortical areas (high-order association areas in the frontal and parietal cortices) and to paralimbic and limbic cortices (parahippocampal gyrus and entorhinal cortex at its temporal termination). Our anatomical study confirms the presence of extensive U-fibre connections between the cingulum and mesial frontal and parietal cortex, along with projections to the parahippocampal region. James Papez (Papez 1937) was the first to stress the importance of the cingulum in describing an anatomic circuit for the processing of emotions. In Papez's limbic circuit, the cingulum represents the external ring, connecting the cingulate gyrus with the hippocampus, while the fornix represents the internal ring, connecting the hippocampus with the mammillary body, that are eventually connected to the anterior thalamus via the mammillothalamic tract of Vicq d'Azir and from here back to the cingulate gyrus. From the initial observations of Papez, the fibre system of the cingulum has been implicated in a variety of emotional and behavioural responses, that may play a role in depression and anorexia nervosa. Complex motor behaviours (Talairach 1973), emotional coloring of sensation and nociception and avoidance behaviour (Pribram and Fulton 1954) have been linked to the anterior cingulate region and cingulum bundle. Modern studies have also shown an abnormality in fractional anisotropy of the cingulum in patients affected by psychiatric disorders, such as schizophrenia (Kubicki 2003), obsessive compulsive disorders (Szeszko 2005) and major depression (Korgaonkar 2014). Of interest, the largest U-fibres connection identified in the present study was between the cingulum and the mesial aspect of the superior parietal lobe (precuneus). In anorexia nervosa, projection to the parietal lobe and parietal lobe dysfunction have been implicated in the

generation of an altered body representation, which is one of the main features of the disease (Favaro 2012, Nico 2010). In summary, the cingulum bundle appears to be an essential “dorsal” limbic pathway connecting the frontal, parietal, cingulate and ventral temporal cortices (Schmamman and Pandya 2009).

The second large associative bundle identified in our study and intimately related to the SCG region is the uncinate fasciculus, connecting the rostral temporal regions with the medial and orbital cortices. Our results are in accordance with previous description of this bundle, that runs in the anterior third of the temporal stem (Martino 2010a). This tract has been considered as the essential component of a “ventral” limbic pathway (Schmamman and Pandya 2009). The anterior temporal lobe is involved in processing modality-specific informations, such as auditory (rostral superotemporal gyrus), visual (rostral inferotemporal region) gustatory (rostral insular opercular cortex) and emotional (amygdala) informations. The orbitofrontal cortex appears to be involved in the emotional response to these stimuli along with self-regulation and decision-making (Bechara 2004). The uncinate fasciculus therefore connects temporal areas that contain modality-specific and multimodal information with frontal areas that regulate behaviour and emotional response to these stimuli.

A third group of fibres described in our dissections are directed from the SCG towards medial prefrontal areas, including Brodmann areas 11,12 and 32. Studies on the primate demonstrated that neurons in the orbitofrontal cortex encode economic value, playing a major role in value assignment underlying economic choices (Padoa-Schioppa 2006). Functional neuroimaging studies in humans have similarly implicated these areas in the

perception of pain and perceived unpleasantness (Rainville 1997), detection of pleasant and unpleasant emotions (Lane 1997) and reward-based decision making (Rogers 1999, Ernst 2004). This last aspect in particular has been associated with addiction behavior, and the medial prefrontal cortex has been proposed as a target for DBS in addiction (Luigies 2012). Of interest, an alteration in reward-based decision making and addiction behavior have been described in both major depression and anorexia nervosa.

A small contingent of fibres has been also observed in the present study between the fronto-polar area and the ventral striatum, seat of the nucleus accumbens. The nucleus accumbens has been considered to play a central role in the reward circuitry, although its role as an interface between limbic and motor system should be also stressed (Floresco 2015). fMRI studies have shown repeatedly that receipt of rewards increases BOLD responses in the nucleus accumbens (Cho 2012). The nucleus accumbens has been implicated in circuitry of anorexia nervosa and depression (Lipsman 2015). DBS of the nucleus accumbens has been proposed for a number of psychiatric conditions, including addiction, Tourette syndrome and depression (Robison 2012).

The SCG white matter and DBS for neuropsychiatric disorders

The idea that white matter structures constitute a functional target is not new in neurosurgery. From the very beginning of psychosurgery, the work of pioneers like Edgas Moniz in Europe and Freeman and Watts in the United States focused on the lesioning of the white matter connections of the frontal lobe for the treatment of psychiatric disorders. Frontal leucotomy (Moniz 1936) or lobotomy (Freeman 1942, Freeman 1950) were

performed in an attempt to ameliorate the symptoms of diverse psychiatric conditions, like schizophrenia and major depression. Nonetheless, an increasing amount of criticism and skepticism towards these procedures was caused by the inconsistent clinical results obtained, the non-negligible burden of complications (Tooth 1961) and – in many cases – by the unregulated practice of frontal lobotomies (Robison 2012). This led to the progressive abandon of prefrontal leucotomies and to the need of selecting more specific targets, tailored on the individual pathology. The American neurosurgeon Scoville initially proposed a “selective undercutting” of the white matter of the frontal lobes, as a less invasive approach compared to the extensive lobotomies popularized by Freeman (Scoville 1949). However, it was the advent of stereotactic neurosurgery that made it possible to tailor more refined lesions of the white matter tracts in psychosurgery. Subcaudate tractotomy (Knight 1964, Knight 1965), anterior cingulotomy (Ballantine 1967, Ballantine 1987) limbic leucotomy (essentially combining subcaudate tractotomy and anterior cingulotomy) (Kelly 1973), and anterior capsulotomy (Talairach 1949) were developed for the surgical management of depression and obsessive-compulsive disorders. In recent years, the development of DBS, initially for the management of movement disorders (Benabid 1989) has introduced the possibility of neuromodulation in the field of psychosurgery, spurring a new interest in this field of functional neurosurgery. DBS has been proposed with success by different groups for the treatment of obsessive-compulsive disorders, with the subthalamic nucleus (Mallet 2008), nucleus accumbens (Greenberg 2006) and anterior limb of the internal capsule (Nuttin 2003) identified as potential targets. More recently, DBS of the SCG has been introduced for treatment-resistant depression and anorexia nervosa (Lozano 2008, Lipsman 2013). The SCG has been selected as a target for these psychiatric disorders on the basis of functional neuroimaging studies that demonstrated an altered metabolism in this region in affected

patients. Metabolic studies with PET showed a decrease in glucose metabolism in patients receiving pharmacological treatment for depression (Mayberg 2000, Drevets 2002). The advantage of DBS versus lesioning is that DBS can modulate the activity of the structures involved (Robison 2012), whether these are grey matter or white matter structures. In the context of movement disorders, stimulation of white matter fibres of the Zona Incerta and Forel's fields has been proposed to explain the clinical efficacy of DBS of the Subthalamic nucleus in ameliorating the symptoms of Parkinson's disease (Vergani 2007). In the same way, stimulation of the white matter fibres of the SCG can play a role in controlling symptoms of depression and anorexia nervosa by modulating the network involved by SCG connections (Mayberg 2005, Hamani 2011, Lipsman 2014).

The results of our study show that the SCG white matter is at the centre of a network involving limbic, prefrontal, and mesiotemporal areas. These information are of potential clinical interest in refining the targeting for SCG DBS. A lesion analysis for limbic leucotomy showed that the structures lesioned in patients who favorably responded to limbic leucotomy correspond largely to the SCG connections described in the present paper, including the cingulum, the uncinate fasciculus, the nucleus accumbens, the medial orbitofrontal cortex and the amygdala (Yang 2014). In a previous multicenter study of subcallosal area DBS for treatment-resistant depression, a minimal variability in the location of the active contacts in the white matter deep to the subgenual gyrus has been reported among participating centres (Lozano 2012). This suggests that stimulation of a relatively small area of white matter at the crossroad of all the connections described in the present paper, is crucial to the clinical effect observed in patients. A more recent study used probabilistic tractography to delineate the white matter pathways mediating successful DBS of the SCG in patients with treatment-resistant depression (Riva-Posse, 2014). The results of this study again show a large correspondence with the findings of

the present investigation, with the cingulum, uncinate fascicles and forceps minor playing a role in mediating DBS for treatment-resistant depression. In addition, our study also showed the presence of connections to the nucleus accumbens, that has also been suggested as a target in treatment-resistant depression (Rigoard 2011).

It seems therefore possible to conclude that modulation of these fibres can contribute to the clinical benefit observed after DBS of the SCG. Knowledge of the white matter connections deep to the SCG, as assessed on preoperative DTI imaging, can help in guiding the implantation of the DBS electrodes in the individual patient. In a recent study, targets within the SCG selected using DTI appeared to be significantly different in location from those selected using conventional T2 sequences. This was considered to have the potential to enhance treatment outcome by reducing the impact of interindividual variability (Bhatia 2012).

3.4.3. Conclusions

The white matter fibres deep to the SCG are at the center of a large network, connecting prefrontal, limbic and mesotemporal regions. Two large associative bundles related to the limbic system, the cingulum and the uncinate fasciculus, are part of the SCG network. Short intralobar fibres connect the SCG to the fronto-polar region and the fronto-polar region to the accumbens. Neuromodulation of these fibres can help to understand the clinical effect in neuropsychiatric disorders such as depression and anorexia nervosa.

FIGURES

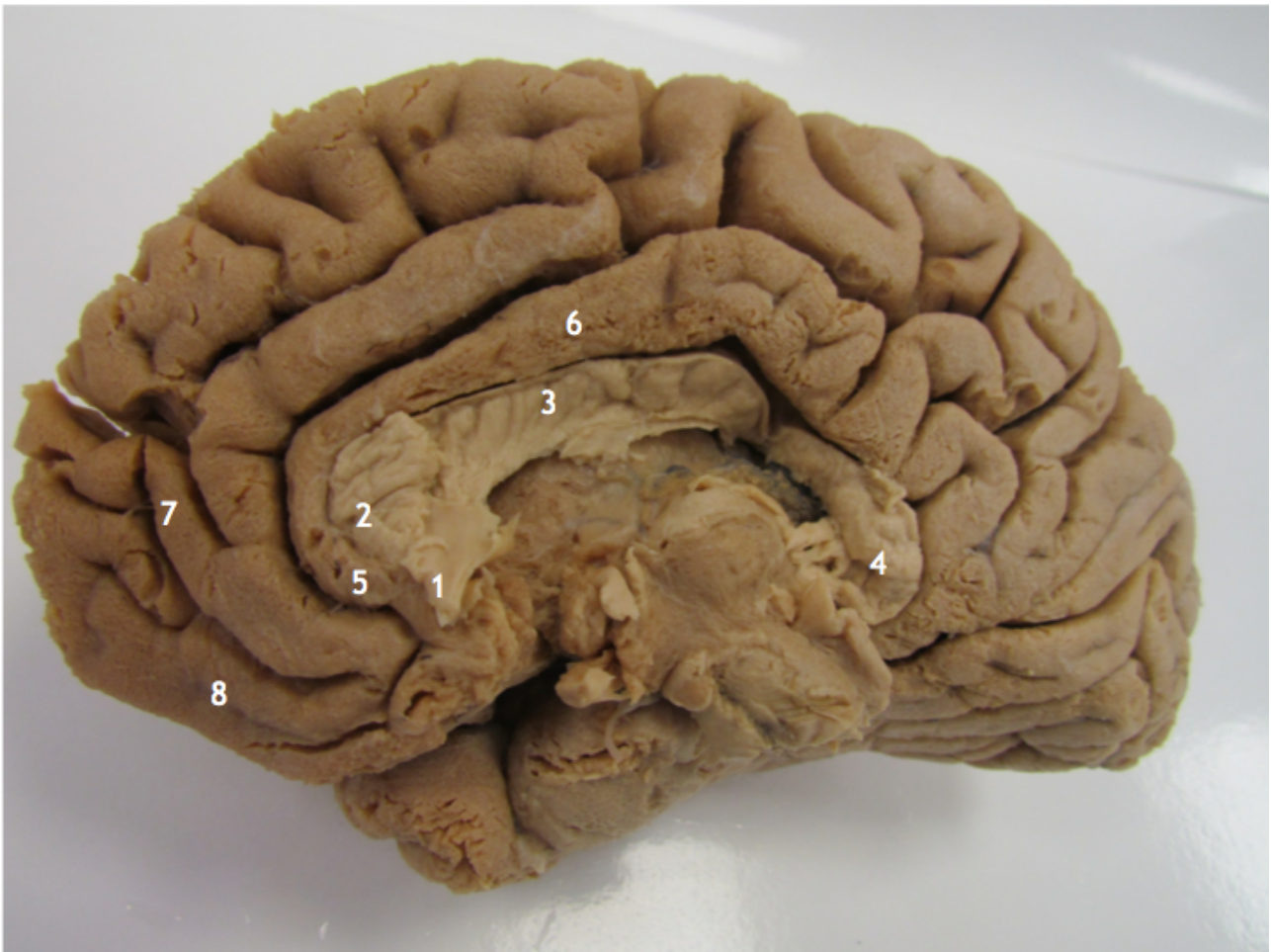


Figure 4.1. Mesial surface of a right hemisphere prepared according to the Klingler technique. 1: rostru; 2: genu; 3: body; 4: splenium of corpus callosum; 5: SCG, 6: cingulate gyrus; 7: fronto-polar region; 8: gyrus rectus

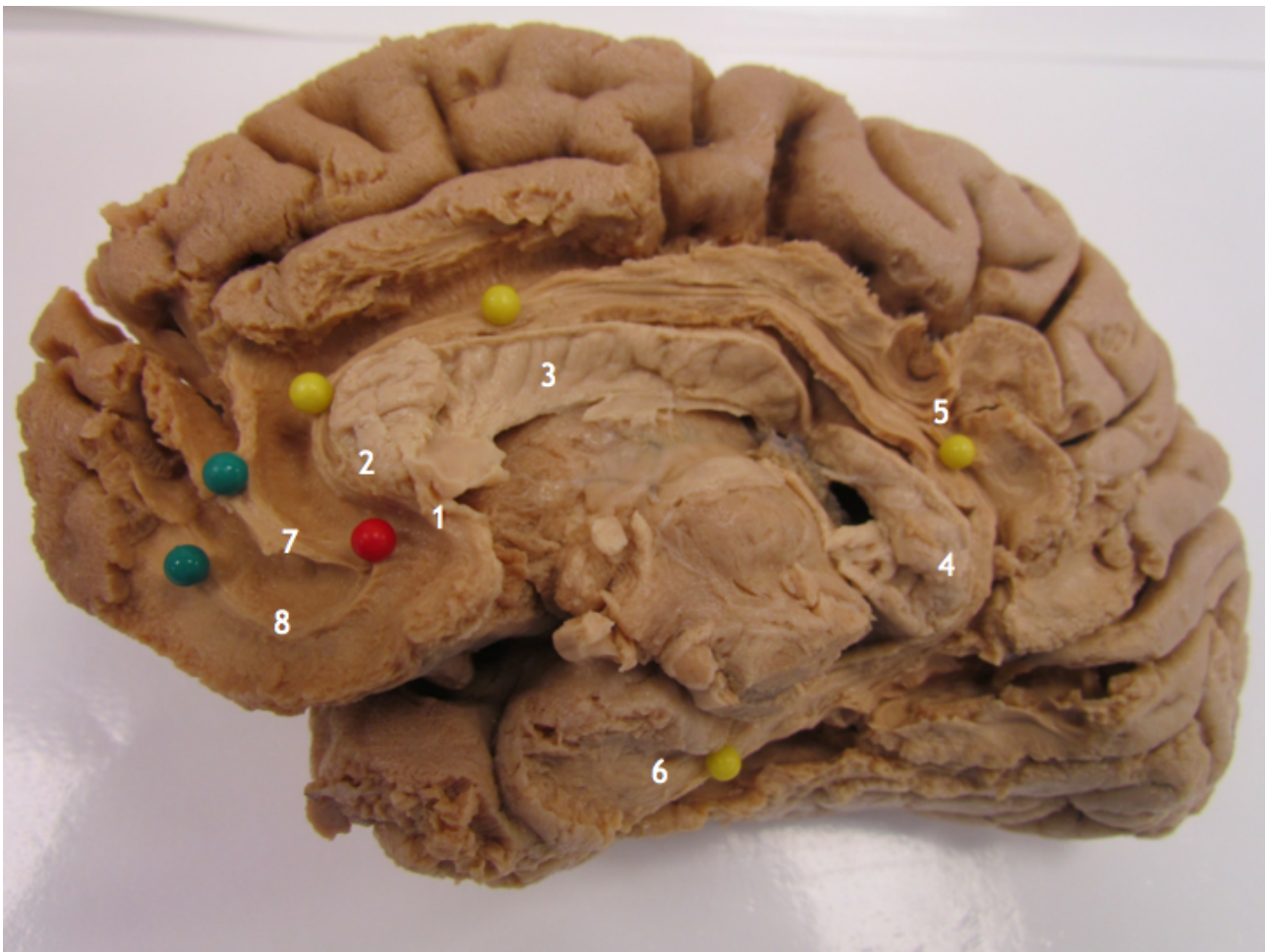


Figure 4.2. Exposure of the cingulum (yellow pins) and fronto-polar fibres (green pins) after removal of the cortex. 1: rostru; 2: genu; 3: body; 4: splenium of corpus callosum; 5: fibres of the cingulum directed to the mesial part of the parietal lobe (precuneus); 6: temporal termination of the cingulum at the level of the mesial parahippocampal gyrus; 7-8: fronto-polar connections. Red pin: white matter deep to the SCG.



Figure 4.3. Dissection performed inferiorly to the SCG to expose the uncinate fasciculus, running from the orbito-frontal to the mesio-temporal region (pink pins). 1: rostru; 2: genu; 3: body of corpus callosum; 4: striatum; 5: posterior portion of the gyrus rectus (partially removed), 6: antero-mesial portion of the temporal lobe. Red pin: white matter deep to the SCG.



Figure 4.4. Dissection completed medially. 1: rostrum; 2: genu; 3: body of corpus callosum. 4: ventral striatum; 5: callosal fibres (forceps minor) exposed after removing the superficial layer of cortex and fronto-polar fibres (6). The accumbens-frontal fascicle, running from the fronto-polar region to the ventral striatum is also exposed (white dots to outline the course of the fascicle. Black pin to show the termination at the level of the striatum). Red pin: white matter deep to the SCG.

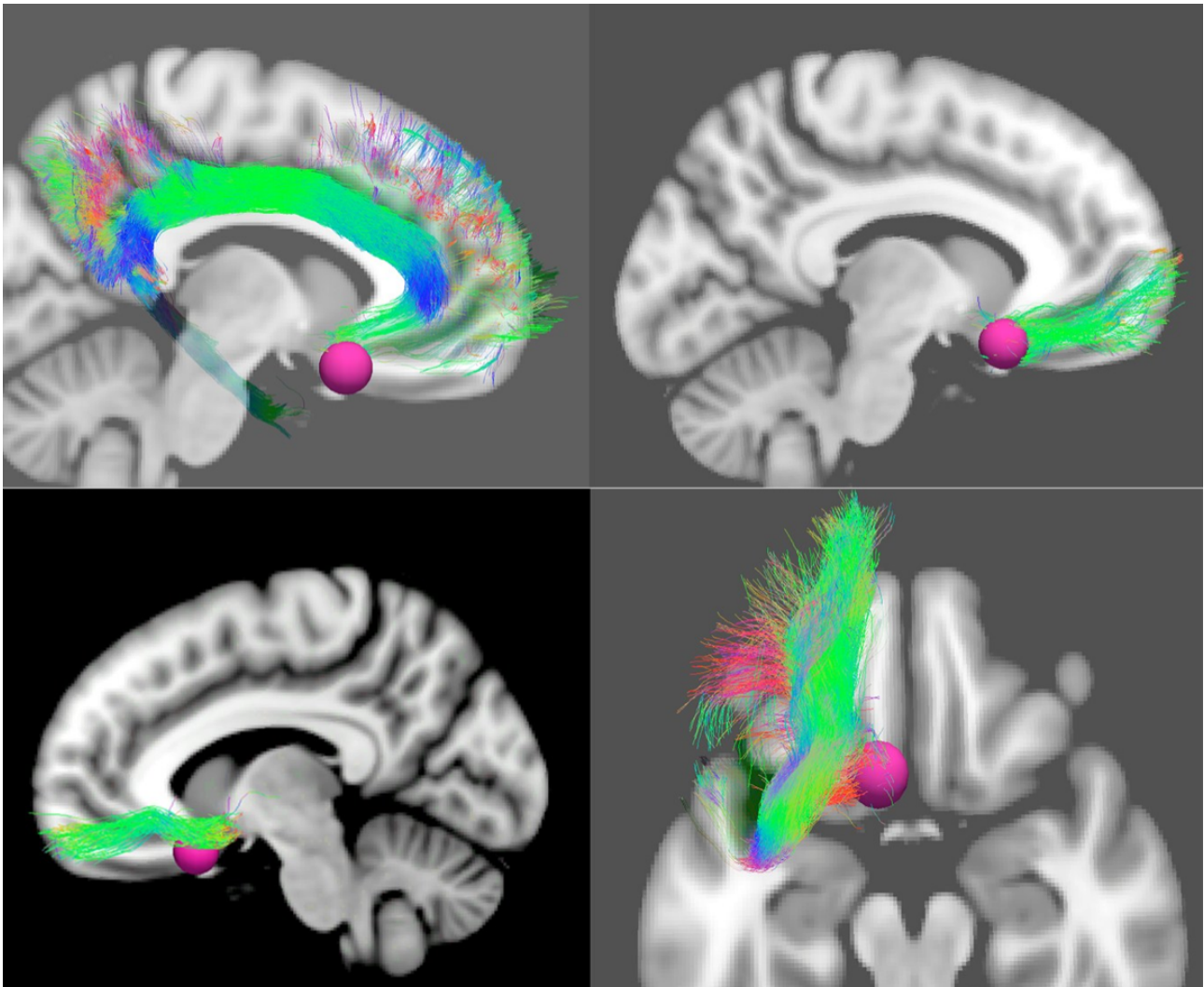


Figure 4.5. Results of advanced tractography. Upper left: delineation of the cingulum, running between the SCG and the parahippocampal gyrus; upper right: fronto-polar fibres, running from the fronto-polar region and the SCG; lower left: fronto-accumbens fascicle, running deeper to the fronto-polar fibres and traversing the SCH white matter to terminate at the level of the accumbens, lower right: uncinate fasciculus, connecting orbito-frontal and mesio-temporal regions

Chapter 4. Discussion

4.1. General discussion

The Klingler technique for white matter dissection is a relatively old method to investigate the anatomy of the white matter fibres. Nonetheless, there has been recently a revived interest in this method within the neurosurgical community, as witnessed by the numerous publications (mostly by neurosurgeons) on white matter dissection.

As seen in the historic paper on Raymond de Vieussens, different techniques for white matter dissection have been elaborated through the centuries. Earlier attempts at dissection of fresh specimens were very difficult, and the anatomical findings confined to large and more easily recognisable bundles, such as the corpus callosum (described by Vesalius). Vieussens adopted the scraping method of dissection used by Willis, and improved the technique of Malpighi of boiling the brains, using oil instead of water. This allowed him to provide a more detailed description of white matter tracts, including the centrum ovale and the cortico-spinal tract. However, it is with the development of fixation techniques (initially alcohol, then formalin) that more refined description of the white matter bundles were provided, along with the first detailed atlases (such as the ones made by Vicq D'Azir or Dejerine). The main advantage introduced by Klingler in the XIX century is the freezing of specimens previously fixed in formalin. This allows the researcher to separate more easily the tracts of fibres after thawing the specimen (Klingler 1936, Ludwig and Klingler, 1956).

Since after its development, the Klingler method has been used as a tool to investigate the white matter anatomy. New anatomical findings have been reported with the use of this technique, along with a better anatomical definition of some tracts. For example, the

course of the Inferior Fronto-occipital fasciculus, with its posterior and anterior terminations, has been clearly described with post-mortem dissection studies (Martino, 2010b, Sarubbo 2013). In the paper on the intralobar occipital fibres, we describe a previously unrecognised white matter tract running anteriorly from the inferior portion of the cuneus to the supero-anterior portion of the lingula. Due to its peculiar shape, the name “sledge runner” has been proposed for this new tract. It is likely that Sachs, in his original atlas on occipital lobe connections, missed this fascicle because in his work only coronal sections were performed. The “sledge runner” is a thin fascicle, with a postero-anterior course in the sagittal plane, and can therefore be either lost or confused with the other U fibers running in the calcarine fissure in the coronal plane. We speculate that this tract, connecting the superior edge of the calcarine fissure and the anterior portion of the lingual gyrus (where the occipital lobe and the parahippocampal gyrus blend together) can be seen as a tract for the integration of visual stimuli from the inferior portion of the visual field and parahippocampal areas involved in the recognition of places (Arcaro, 2009).

Another application of the Klingler technique is to complement findings provided by tractography. The major advantage of tractography techniques is the possibility to investigate white matter tracts *in vivo* (Catani 2008b). Shortcomings of tractography include its limited resolution in following the fibres to their cortical termination (as this can only be inferred from the location and orientation of the average tract end points) and in discriminating between different fibres tracts when they run in close proximity to each other (Catani 2003). In two papers (the one on the connections of the SMA and the one on the connections of the SCG) we integrated the post-mortem findings obtained with Klingler technique with the results provided by advanced diffusion tractography. A good concordance was found between the two methods. All the connections described in the two papers were identified with both techniques, with similar trajectories and directions of

the fibre tracts observed. Post-mortem dissection was able to clearly identify the cortical termination of the bundles, showing the relationship of the fibres to other white matter tracts and to adjacent cortical landmarks. Advanced tractography had the advantage of obtaining quantitative data about the lateralisation of specific tracts for intra and inter-individual comparison. In this regard, the two techniques can be seen as complementary tools to strengthen the validity of anatomical findings.

From a neurosurgical perspective, one of the main advantages of white matter dissection is that it can provide a unique 3D view and representation of white matter tracts, enabling the surgeon to have a better understanding of the anatomy of a specific tract in relation to other tracts or cortical areas (Martino 2010b). The direct clinical application is in the identification of essential connections for specific function during surgery for removal of brain tumour. In recent years, several authors have reported the use of subcortical white matter stimulation to identify tracts involved in language (Duffau 2008), visuospatial attention (Thiebaut de Schotten 2011), movement (Keles 2004) and vision (Peltier 2006). The improved knowledge of white matter anatomy, along with the use of subcortical stimulation, has been therefore used by neurosurgeons as an adjunct to maximise the extent of resection of brain tumours while minimising the risk of inducing neurological deficits by sparing essential connections of specific networks. In the paper on the intralobar fibres of the occipital lobe, we stressed the importance of the fibres forming the stratum sagittale. Semantic paraphasia have been induced by stimulation of these fibres during awake craniotomy for removal of gliomas involving the occipital lobe and extending towards the temporal lobe (Viegas 2011). The resection was therefore stopped at this level in order to avoid language deficits, with the stratum sagittale representing the functional limit for surgery involving the occipital lobe. In the paper on the SMA connections, we highlighted the role of the SMA connectivity to explain the genesis of the so called “SMA

syndrome”, originally described by Laplane (Laplane 1977). Connections to motor areas, including U-shaped fibres to the primary motor cortex and fibres to the striatum, can explain the motor symptoms observed after surgical removal of tumours involving the SMA, including akinesia and lack of bimanual coordination. The frontal aslant tract, connecting the pre-SMA with the inferior frontal gyrus, can explain the onset of mutism or speech reduction (“transcortical motor aphasia”) seen after surgery involving the dominant SMA. Knowledge of these fibres can help to predict the postoperative course of patients with tumours in the SMA. This is important for preoperative patient counselling and also to plan an individualised postoperative rehabilitation. Finally, in the article on the SCG connections, we suggested to apply our anatomical results to refine the electrode positioning in DBS for neuromodulation of the SCG region in psychiatric disorders. The use of preoperative tractography can yield detailed data about the individual white matter anatomy of patients, which can be incorporated in the surgical planning for the implantation of DBS electrodes. This was considered to have the potential to enhance treatment outcome by reducing the impact of interindividual variability (Bhatia 2012).

4.2. Future directions of research

The next step in my research will be to translate the anatomical knowledge gathered with my PhD into my clinical practice as a neurosurgeon. In the last year, I have completed a surgical neuro oncology fellowship at King's College London, where I specialised in the treatment of brain tumours. Gliomas (brain tumours of glial origin) are the most common primary brain tumours, with approximately 3,700 new cases/year in the UK. Of these, 20% are low grade gliomas, which tend to affect younger patients, have a better prognosis and characteristically tend to affect eloquent areas of the brain (Duffau, 2004). An effective neurosurgical treatment aimed at maximal resection is key to extend survival and preserve quality of life. Nonetheless, tumours affecting eloquent areas (motor and language areas, in particular) represent a challenge to the neurosurgeon, since tumour removal is at risk of inducing unacceptable deficit and permanent disability. In addition, studying these patients with intraoperative cortical and subcortical mapping represent a unique opportunity to improve our understanding of brain anatomy and function.

In this future research I propose to capitalise on the knowledge of white matter anatomy obtained during my PhD to better understand the organisation of human language and movement, with the view to investigate mechanisms of compensation and recovery in patients with brain tumour.

A prospective longitudinal cohort study design will be adopted for this study. Patients with brain tumours in the close proximity of eloquent areas will be recruited by the neuro-oncology Multi-Disciplinary Team (MDT) through the neuro-oncology clinic at King's College Hospital. Patients will be assessed in three stages:

- 1) Preoperative neurological assessment and identification of the cortical and subcortical eloquent regions and white matter pathways by combining fMRI and diffusion tractography.

- 2) Intraoperative cortical and subcortical stimulation for the anatomical and functional validation of functional networks, in particular with identification of the white matter tracts involved in language and movement. The information obtained during surgery will be used to maximise the extent of tumour resection while sparing essential language functions.
- 3) Postoperative neurological assessment, fMRI and diffusion tractography will be used to identify anatomical and functional changes that occur after surgery (including compensatory re-organisation) and correlation with clinical outcomes.

The primary goal is to define the cortical hubs and nodes of functional networks and the major pathways that connect them (figure 4.1). The second goal is to determine essential components of the network as defined in the three stages of the project. The third goal is to identify specific mechanisms of functional recovery, especially in relation to individual lateralisation of white matter tracts.

The project will be based at the Neurosurgical Department of King's College Hospital, which has one the largest neuro-oncology practice in the UK, with more than 1400 patients referred each year to our MDT. Integration of neuroimaging with intraoperative stimulation is already an established practice within the Neurosurgical Department. fMRI and tractography will be acquired at the Clinical Research Facilities (CRF) of King's College, London. Neuroimaging will be performed in collaboration with Marco Catani and other members of the Natbrainlab, which have extensive experience in imaging analysis. The combined expertise of the two departments will contribute to the success of the study.

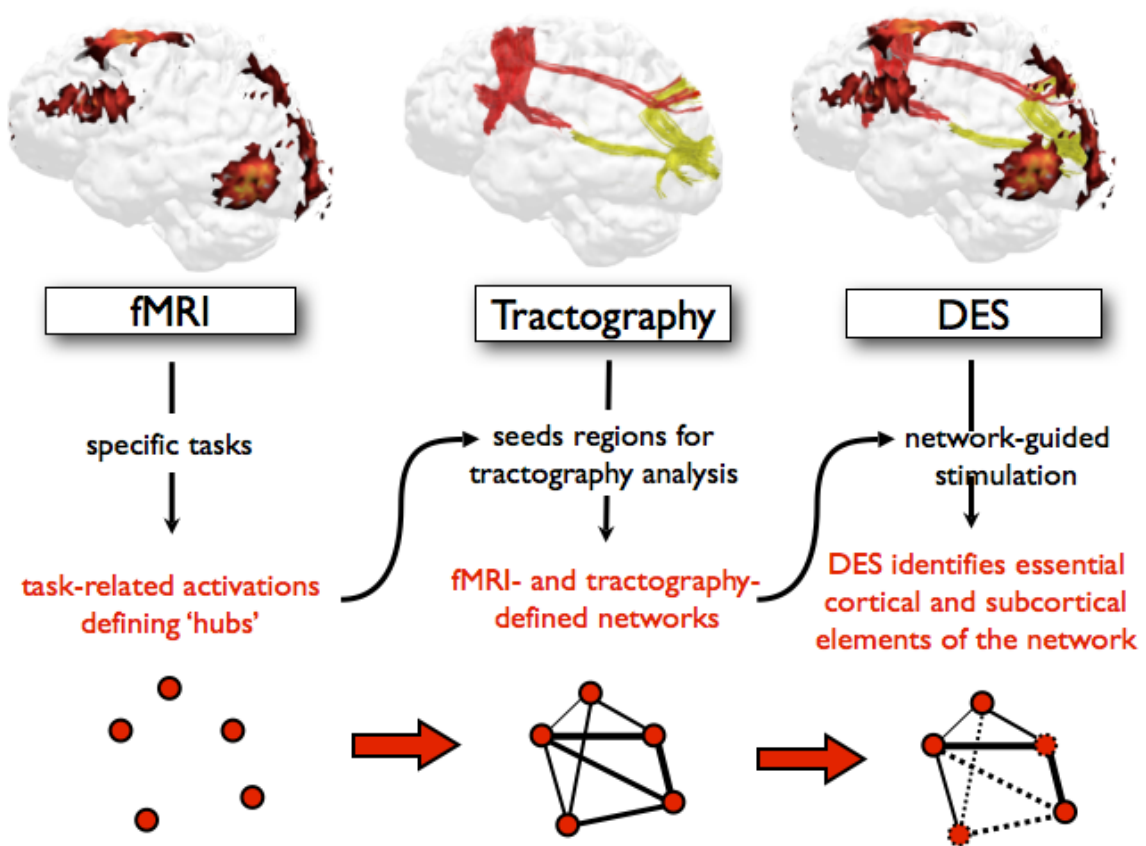


Figure 4.1. Steps in the identification of the functional connectomes as outlined in the research proposal.

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APPENDIX A

(papers published and presented in the present submission)

Raymond de Vieussens and his contribution to the study of white matter anatomy

Historical vignette

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In recent years, there has been a renewed interest in the study of white matter anatomy, both with the use of postmortem dissections and diffusion tensor imaging tractography. One of the precursors in the study of white matter anatomy was Raymond de Vieussens (1641–1716), a French anatomist born in Le Vigan. He studied medicine at the University of Montpellier in southern France, one of the most ancient and lively schools of medicine in Europe. In 1684 Vieussens published his masterpiece, the *Neurographia Universalis*, which is still considered one of the most complete and accurate descriptions of the nervous system provided in the 17th century. He described the white matter of the centrum ovale and was the first to demonstrate the continuity of the white matter fibers from the centrum ovale to the brainstem. He also described the dentate nuclei, the pyramids, and the olivary nuclei. According to the theory of Galen, Vieussens considered that the function of the white matter was to convey the “animal spirit” from the centrum ovale to the spinal cord. Although neglected, Vieussens’ contribution to the study of white matter is relevant. His pioneering work showed that the white matter is not a homogeneous substance, but rather a complex structure rich in fibers that are interconnected with different parts of the brain. These initial results paved the way to advancements observed in later centuries that eventually led to modern hodology. (<http://thejns.org/doi/abs/10.3171/2012.8.JNS12387>)

KEY WORDS • history of medicine • hodology • Vieussens • white matter • neurosurgical history

IN recent years, there has been a growing interest in the study of white matter anatomy. The development of diffusion tensor imaging for in vivo tractography^{5,15,18} and the renewed interest in postmortem dissections, usually performed by neurosurgeons,^{14,22,29} have both contributed to this evolving branch of neuroanatomy. Hodology, the study of white matter connectivity, is of great importance, not only for a better understanding of brain functioning but also to tailor the surgical approach to the individual functional anatomy of each patient.⁸

This article focuses on the pioneering work in neuroanatomy, and white matter anatomy in particular, performed by Raymond de Vieussens (1641–1716), a French anatomist and medical doctor from the University of Montpellier. His first publication, the *Neurographia Universalis*,³² is still regarded as one of the most important contributions to the field of neuroanatomy of the 17th century. Surprisingly little attention has been paid to the historical role of Vieussens,⁷ and there is no readily available study in the literature dedicated to reviewing his contribution to the study of white matter anatomy.

The Life and Times of Raymond de Vieussens

Raymond de Vieussens was born in 1641 in the city

of Le Vigan in southern France (Fig. 1). There is, however, some uncertainty regarding his birth, with some authors citing 1635 as his year of birth.^{13,19,25} He studied philosophy at Rodez in France before moving to Montpellier, where he embraced anatomical research and medicine. He completed his studies and graduated in medicine from the University of Montpellier in 1670. After graduation, he was appointed as physician to the Saint-Eloi hospital in Montpellier. There, he devoted “ten years of hard work” to the study of the nervous system that eventually led to the publication of the *Neurographia Universalis* in 1684.³² This first work was well accepted by the European medical community as one of the most complete descriptions of the nervous system of his time. It also brought Vieussens recognition both in France (where he was appointed a member of the Academy of Sciences of Paris) and abroad (he became a fellow of the Royal Society of London). A few years later, in 1688, he was introduced to King Louis XIV, whose long reign formed the background of Vieussens’ entire life. It seems that he was highly appreciated by the royal family.^{19,25} The king himself granted Vieussens an annual pension of 1000 livres, leaving him at the same time the freedom of continuing to practice the medical profession. A cousin of Louis XIV, the Duchess of Montpensier,



FIG. 1. Portrait of Raymond de Vieussens. The inscription around the portrait says: "Raymond de Vieussens, medical doctor from Montpellier, aged 42." From *Neurographia Universalis*, 1684, originally published by Joannes Certe.

appointed Vieussens as her personal physician. During the following years, Vieussens continued to work at Saint-Eloi Hospital, where he developed an interest in the study of heart anatomy and cardiology. This led to a series of important works. In 1705 he published the *Novum vasorum corporis humani systema* ("New vessels of the human body"), where he gave an accurate description of the lymphatic and blood vessels of the heart.³³ In particular, he was the first to describe small ducts joining the ventricular cavities to the coronary vessels. Vieussens called these channels "ducti carnosus" (fleshy ducts); as we know today, these channels correspond to the capillaries of the coronary arteries. The other major work on cardiology was the *Traité nouveau de la structure et des causes du mouvement naturel du coeur* ("New treatise on the structure of the heart and the causes of its natural motion"), published in 1715, 1 year before his death.³⁴ In this work Vieussens described in detail the pericardium, coronary vessels, and muscle fibers of the heart. He also described clearly the clinical picture and the findings at autopsy of patients with mitral valve stenosis and aortic insufficiency.^{16,19} This account was one of the major contributions to the understanding of the pathophysiology of these two clinical conditions.

Vieussens lived in 17th-century France when his country, under the rule of Louis XIV, became one of the

leading powers in Europe. The historical period when Vieussens studied and worked was also marked by the birth of the scientific method as we conceive it today, based on the accurate observation of natural phenomena and the experimental reproducibility of these observations to explain the laws of nature. A generation before Vieussens, Galileo set the basis of modern physics, while William Harvey provided us with the method of physiology. A contemporary of Newton, Leibniz, and Locke, Vieussens shared the scientific spirit of his time.

The Medical School of Montpellier: a Rich Cultural Milieu

The Medical School of Montpellier, in southern France, is one of the most ancient medical schools in Europe, second only to the Schola Salernitana (School of Salerno) in Italy.¹⁰ Medicine was being taught in Montpellier as early as 1181, when Gilhem VIII, lord of the city, established the right of teaching medicine. It was in 1289 that the University of Montpellier was officially founded and recognized by Pope Nicholas IV with the bull "Quia sapientia." The newly created university incorporated the Schools of Medicine, Theology, and Law and Letters. During the Middle Ages, the School of Medicine increased its reputation, combining the teaching of the Jewish and Arabic traditions and attracting students from all over Europe. Important figures of the time were Arnaud de Villeneuve (1240–1311), physician, alchemist, and theologian, and Gui de Chauliac (c. 1300–1368), physician, surgeon, and author of a well-known treatise on surgery in Latin titled *Chirurgia Magna*.⁹ During the Renaissance the faculty of medicine acquired more independence from the Church, coming progressively under the influence of the state and of the King of France. Possibly because of this autonomy, cadaver dissections were routinely carried out in Montpellier as part of the teaching of human anatomy. This is confirmed by the building in 1556 of the first anatomical amphitheater in France dedicated to the dissection of cadaveric specimens. The use of human cadavers for anatomical studies was a well-established practice in Montpellier, as witnessed by Vieussens himself. In the introduction to the *Neurographia Universalis*, Vieussens declared that the aim of his medical profession was to investigate the causes of diseases by means of "careful dissections of human cadavers, of which I had large availability."³²

Study of White Matter Anatomy Before Vieussens: on the Shoulders of Giants

Andreas Vesalius (1514–1564), the father of modern anatomy, was the first to differentiate between the gray and white matter of the brain. In his main work, *De humani corporis fabrica*, he distinguished between the softer and yellowish cortex and the firmer, whiter substance below it.³⁰ He also described in detail the corpus callosum and recognized that it connected the 2 hemispheres. We have to wait a century after Vesalius before the first attempts to dissect and investigate in detail the anatomy of the white matter were made.²⁸ Nicolaus Steno (1638–1686) suggested for the

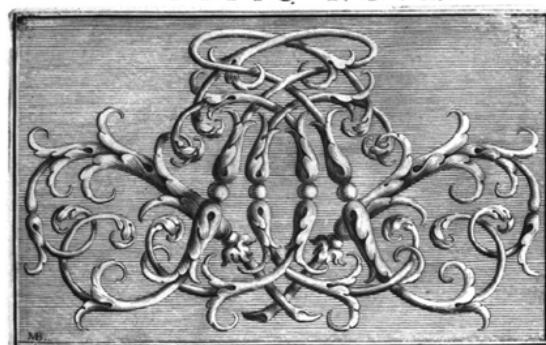
first time to follow “the threads through the substance of the brain to find out where they go and where they end.”⁷ One of the problems at the time was the preparation of the cadaveric specimens, as dissection of the white matter was technically difficult. This was partially overcome by Marcello Malpighi (1628–1686). Boiling the brains in water, he was able to trace the white matter bundles of the brain and cerebellum. He was also the first to show, using a microscope, that the white matter is organized in fibers. After Malpighi, Thomas Willis (1621–1675), working in Oxford, developed a technique of scraping the white matter to demonstrate the bundles and the intricate arrangements of the fibers. Both Malpighi and Willis were directly acknowledged by Vieussens in his *Neurographia Universalis*.³²

Neurographia Universalis

The *Neurographia Universalis, hoc est omnium corporis humani nervorum, simul et cerebri, medullaeque spinalis descriptio anatomica* (“General Neurography, that is, the anatomical description of all the nerves of the human body, and also of the brain and spinal cord”) was published in Lyons in 1684 by the editor Joannes Certe (Fig. 2).³² This edition was enriched by beautiful copperplate illustrations created by Jacques Beaudou, who was one of the foremost engravers in Montpellier (Fig. 3). The treatise is divided in 3 parts: the first volume is dedicated to the brain, the second to the spinal cord, and the third to the nerves (both intracranial and peripheral). From a methodological point of view, Vieussens adopted the scraping method of dissection used by Willis, and improved the technique of Malpighi of boiling the brains, using oil instead of water.²⁸ In Chapter 10 of the first volume titled “De distinctis duabus cerebri substantiis” (“the two distinct substances of the brain”), Vieussens clearly differentiated between white and gray matter, highlighting the different texture between the two: “The brain is composed of two substances, one different from the other: the gray and the white matter; the gray, if observed in its natural state or after boiling in water or oil, appears to be much softer than the white matter.” After heating the brains in oil, he was also able to demonstrate that “the white matter of the brain...is formed by innumerable fibers connected together, and arranged in multiple fascicles; this appears evident after it is boiled in oil.” In Chapter 11, “De cerebro stricto sumpto” (“the brain considered in strict sense”), Vieussens described the hemispheres and the convexity of the brain. He gave a detailed account of the corpus callosum (for which he proposed the name of “verum fornix”), identifying it as a white matter structure connecting the 2 halves of the brain. He then illustrated the centrum ovale, the oval-shaped white matter lying beneath the cortex and surrounding the corpus callosum and the ventricle walls, as demonstrated in “tabula VI” of the first volume (Fig. 4). The introduction of the term “centrum ovale” is one of the legacies to the field of neuroanatomy left by Vieussens. The method followed by Vieussens in his description of the brain anatomy is of a “top to bottom” dissection. Starting from the plane of the centrum ovale, he pursued the dissection inferiorly, exposing the lateral ventricles (with the foramen of Monro, termed “vulva” by Vieussens), the septum pellucidum, the fornix, the third ventricle, and the

RAYMUNDI VIEUSSENS DOCTORIS MEDICI MONSPELIENSIS NEUROGRAPHIA UNIVERSALIS.

HOC EST,
OMNIUM CORPORIS HUMANI NERVORUM,
simul & cerebri, medullaeque spinalis
DESCRIPTIO ANATOMICA;
EAQUE INTEGRA ET ACCURATA, VARIIS
Iconibus fideliter & ad vivum delineatis, aequae incisae illustrata:
CUM IPSORUM ACTIONE ET USU,
Phyfico discursu explicatis.
EDITIO NOVA.



LUGDUNI,
Apud JOANNEM CERTE, in vico Mercatorio,
sub signo Trinitatis.
M. DC. LXXXIV.
CUM PRIVILEGIO REGIS.

Fig. 2. Cover of the *Neurographia Universalis*. From *Neurographia Universalis*, 1684, originally published by Joannes Certe.

thalami. At this level, Vieussens described the basal ganglia region, where he found tracts of white matter interspersed in the gray matter of the nuclei. Vieussens adopted the term “corpora striata” (striate bodies) because of the presence of white matter fibers. He distinguished between superior, middle, and inferior striate bodies, which possibly correspond to the caudate and lenticular nuclei (Fig. 5). We note here that the terminology introduced by Vieussens regarding the basal ganglia is somewhat obscure, and perhaps for this reason it was not followed by other authors.⁷ Following the fibers caudally, Vieussens was able to demonstrate, for the first time, the continuity of the white matter through what is known today as the internal capsule, down to the pyramidal tracts and the brainstem (Fig. 6). The later chapters of the first volume are dedicated to the cerebellum and to the so-called medulla oblongata, with the first description of the dentate nuclei, the pyramids, and the olivary nuclei. Vieussens, following Thomas Willis and contrary to modern terminology, used the term “medulla oblongata” to indicate the deep white matter of the hemispheres, the thalami, and brainstem.

Despite this accurate and precise anatomical description, the physiological explanation provided by Vieussens

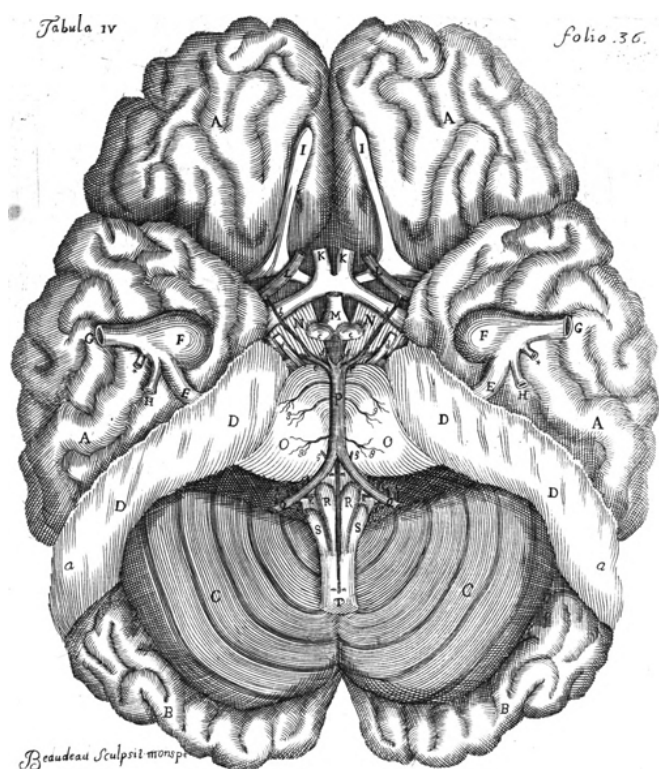


FIG. 3. Illustration from the *Neurographia Universalis*, representing the basal aspect of the brain. It is signed by Jacques Beaudeau (left inferior corner). From *Neurographia Universalis*, 1684, originally published by Joannes Certe.

was still strongly influenced by the theories of the “animal spirit” of Galen. In Viessens’ view, the structural organization of the white matter fibers, running from the centrum ovale to the medulla oblongata and the spinal cord, had the purpose of conveying the animal spirit. “It is possible to see how the animal spirit...which penetrates in the white matter fibers, moves from the anterior regions to the posterior; and through the tracts of white matter that run caudally from the posterior part of the centrum ovale...[the animal spirit] reaches inferiorly the posterior origins of the spinal nerves.”³² Thus, the intricate arrangements of the white matter fibers explain the different range of thoughts and emotions that the animal spirit can generate. “The white matter is formed by long and curved fibers, that are so mixed and interrelated that they take the form of a spongy body, that the animal spirit permeates in multiple, different and inexplicable ways; so that within it [the spirit] undergoes multiple, different and inexplicable emotions; because of their different arrangements, different thoughts are generated in the mind.”³²

The Legacy of Raymond de Viessens

The *Neurographia Universalis* had a great impact on the study of neuroanatomy in Viessens’ time. Along with Thomas Willis’ masterpiece, *Cerebri anatome* (published in 1664), it is considered one of the most complete and accurate descriptions of the nervous system from the 17th century.³⁵ Many investigators continued Viessens’ work. With a technical improvement, obtained by hardening the

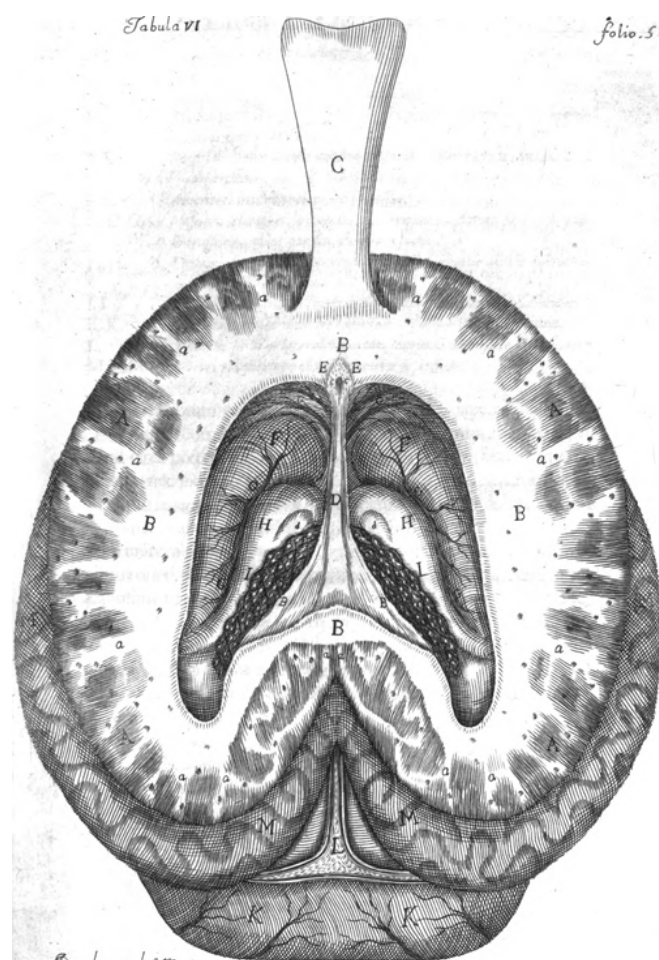


FIG. 4. Illustration from the *Neurographia Universalis*. The human brain is sectioned following the “top to bottom” approach used by Viessens. The gray matter (A) is differentiated from the white matter of the centrum ovale (B), surrounding the ventricles, clearly represented. The corpus callosum (C), sectioned and displaced forward, is also shown. From *Neurographia Universalis*, 1684, originally published by Joannes Certe.

brain in an alcohol solution, Felix Vicq d’Azir (1748–1794) confirmed in his dissections many of Viessens’ findings.³¹ D’Azir also provided a more detailed description of the centrum ovale, which he renamed “centrum semiovale.” More than a century after Viessens’ work, Johann Christian Reil (1759–1813) introduced the term “corona radiata” to describe the centrum ovale.⁷ He clarified the relationship and the continuity between the fibers of the centrum ovale and the internal capsule and cerebral peduncles, at the same time describing the insula and the external capsule.

Surprisingly, the contribution of Viessens has been in part neglected, and he has been rarely cited in the literature. Relatively few authors were involved in the study of white matter pathways during later centuries, notably Karl Friedrich Burdach (1776–1847), Pierre-Louis Gratiolait (1815–1865), and Joseph Jules Dejerine (1849–1917). Dejerine also gave the first account of a disconnection syndrome, describing a case of alexia without agraphia in a patient with a lesion involving the white matter of the splenium of the corpus callosum.⁴ The current standard for white matter dissection in postmortem human

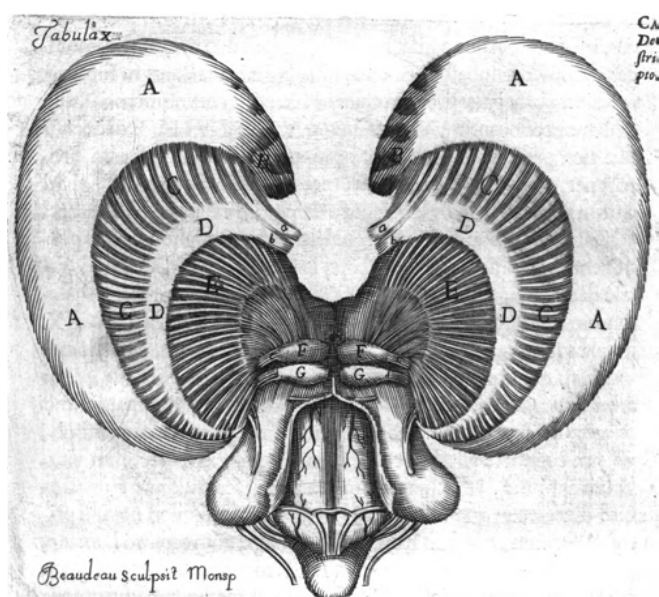


FIG. 5. Illustration from the *Neurographia Universalis*. The white matter projections from the centrum ovale (A) through the corpora striata (C and E) are represented. From *Neurographia Universalis*, 1684, originally published by Joannes Certe.

specimens is the technique developed by Joseph Klingler (1888–1966),^{1,17} who introduced the process of freezing previously fixed brains. The ice that forms between the fibers separates the white matter fascicles, thereby facilitating the dissection. The Klingler method allowed for more accurate anatomical investigations, with the clear identification of the major white matter fascicles. Few authors performed dissections according to this technique until recent years, when a renewed interest in white matter

connections arose in the neurosurgical community.²⁹ In the last decade, several fiber dissection studies have been reported, some of these combined with diffusion tensor imaging, providing original data.^{3,6,14,20,23,24} In particular, the cortical termination of some controversial fascicles has been elucidated.^{21,27} Continuing the clinicopathological work inaugurated by Dejerine, different authors have also investigated the function of white matter pathways, especially using intraoperative subcortical stimulation.^{2,11,12,26} Such findings have allowed for the proposal of new concepts in the organization of the human brain, switching from localizationism to hodotopy, with applications in neurosciences as well as in clinical practice, and particularly in neurosurgery, where sparing important white matter fascicles is vital in preserving function.⁸

One further legacy left by Raymond de Vieussens relates to the relationship between science and philosophy. The methods of Vieussens' anatomical investigation have been improved on but remain comparable to postmortem dissections performed today. The methods available to him for analyzing the functional aspects of the CNS were vastly different from those that have been developed since. In parallel with this distinction between a comparatively stable technology of anatomical investigation and rapidly changing technology of functional investigation is the distinction between Vieussens' anatomical and functional theories. His anatomical theories have been refined but remain broadly compatible with our modern interpretations. By comparison, his functional theories appear to have been surpassed from a modern perspective. In the past, medicine adopted a philosophical explanation for biological processes that could not be otherwise explained (for example, the "animal spirit" of Vieussens and Galen). The change in this viewpoint originated in technological development that only secondarily engendered a philosophical change, rather than the other way around. Vieussens lived in the 17th century, when the modern scientific method was at its very beginning. His interest was in an organ system in which the methods of investigation extend from arguably the most constant to the most changing over the intervening centuries.

Conclusions

Raymond de Vieussens opened the door to investigating the white matter of the brain in humans. His pioneering work showed that the white matter is not a homogeneous and indistinct substance, but rather a complex structure, rich with fibers that are interconnected with different parts of the brain. These initial results paved the way to the advancements observed in later centuries that have subsequently found application in so many fields of neuroscience.

Disclosure

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

Author contributions to the study and manuscript preparation include the following. Conception and design: Vergani, Morris, Mitchell. Acquisition of data: Vergani. Drafting the article: Vergani, Morris, Duffau. Critically revising the article: all authors. Reviewed

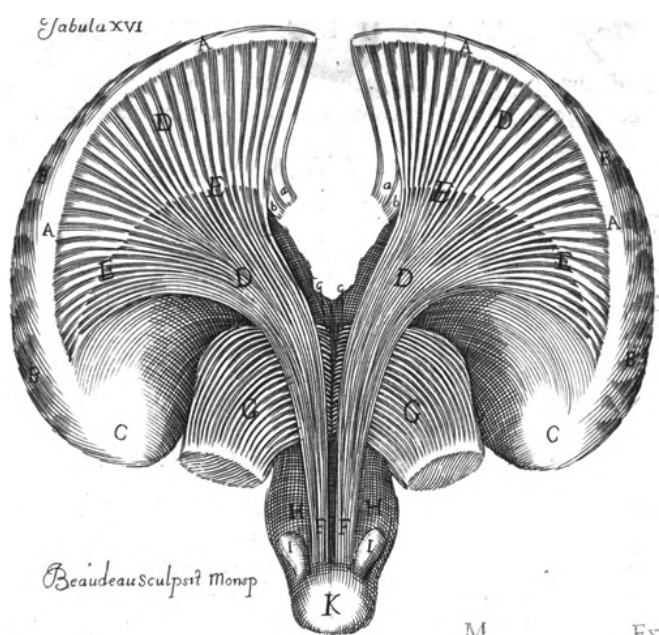


FIG. 6. Illustration from the *Neurographia Universalis*. The continuity of the white matter from the centrum ovale (A) through the medulla oblongata (D) is demonstrated. From *Neurographia Universalis*, 1684, originally published by Joannes Certe.

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Intralobar fibres of the occipital lobe: A post mortem dissection study



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ABSTRACT

Introduction: The atlas by Heinrich Sachs (1892) provided an accurate description of the intralobar fibres of the occipital lobe, with a detailed representation of the short associative tracts connecting different parts of the lobe. Little attention has been paid to the work of Sachs since its publication. In this study, we present the results of the dissection of three hemispheres, performed according to the Klingler technique (1935). Our anatomical findings are then compared to the original description of the occipital fibres anatomy as detailed by Sachs. **Methods:** Three hemispheres were dissected according to Klingler's technique (1935). Specimens were fixed in 10% formalin and frozen at -15°C for two weeks. After defreezing, dissection of the white matter fibres was performed with blunt dissectors. Coronal sections were obtained according to the cuts originally described by Sachs. In addition, medial to lateral and lateral to medial dissection of the white matter of the occipital lobe was also performed. **Results:** A network of short association fibres was demonstrated in the occipital lobe, comprising intralobar association fibres and U-shaped fibres, which are connecting neighbouring gyri. Lateral to the ventricles, longitudinal fibres of the stratum sagittale were also identified that are arranged as external and internal layers. Fibres of the forceps major were also found to be in direct contact with the ventricular walls. We were able to replicate all tracts originally described by Sachs. In addition, a previously unrecognised tract, connecting the cuneus to the lingual gyrus, was identified. This tract corresponds to the "sledge runner", described in tractography studies.

Conclusions: The occipital lobe shows a rich network of intralobar fibres, arranged around the ventricular wall. Good concordance was observed between the Klingler dissection technique and the histological preparations of Sachs.

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The present work is the first contribution to a series of publications dedicated to the investigation of the brain and its functions in health and pathology. This field of research is still heavily under investigated and nearly every contribution to it is a step forward similar to an expedition into unknown territory comparable to the “deepest Africa”.

C. Wernicke, introduction to Sachs’s atlas

Breslau, 1892

1. Introduction

When it was published, the atlas of Heinrich Sachs (*“The hemispheric white matter of the human brain. Part I: The occipital lobe”*, 1892) was saluted by his mentor Wernicke with great enthusiasm. It represented the most accurate description of the intralobar fibres of the occipital lobe ever provided in humans, with a particularly detailed representation of the short associative tracts connecting different portions of the lobe (Fig. 1). At the end of the 19th century, the study of white matter anatomy was revolutionised by the introduction of microscopy and histological preparations of cerebral specimens (Schmahmann and Pandya, 2009). Brains collected at autopsy were fixed, initially using alcohol or chromic acid and only later with formaldehyde. Once the fixation process was completed, thin sections were obtained using the microtome (*“as thin as the microtome allows”* as stated by Sachs). The hereby obtained slices were stained and scrutinised under the microscope, to reveal the complex organisation in layers and fascicles of the white matter. Great neuroanatomists of the time, including Meynert (1833–1892), Flechsig (1847–1929) and Wernicke (1848–1900), applied these novel histological techniques to investigate white matter anatomy (Catani, Forkel, & Thiebaut de Schotten, 2010). Heinrich Sachs, under the tutorage of Wernicke, applied the histological method to the detailed study of the white matter of the occipital lobe. His method consisted of preparing the sections with celloidin, and then treating them with the Pal-haematoxylin and the picrocarmine stains (the former being a specific myelin stain) (Whethered, 1888, Forkel et al., 2014). Surprisingly, little attention has been paid to the work of Sachs after; one reason being that his work was only available in German. The importance of the anatomy and function of the short associative fibres – emphasised in the work of Sachs – has also long been neglected (Catani et al., 2012).

Less than half a century after the publication of Sachs’ atlas, a new technique was developed by Joseph Klingler (1935). Working as an anatomical preparator at the University of Basel (Switzerland), Klingler introduced the process of freezing the brains previously fixed in formalin (Agrawal et al., 2011). The freezing process is a crucial step, since the ice that forms between the fibres separates the white matter fascicles, thus facilitating the dissection. The Klingler method greatly improved the techniques of gross dissection of the white matter of the brain, allowing for more accurate anatomical investigations (Ludwig & Klingler, 1956).

In recent years, the advancement of novel in vivo magnetic resonance imaging (MRI) techniques, such as diffusion

imaging tractography (Catani & Thiebaut de Schotten, 2008; Jones, Simmons, Williams, & Horsfield, 1999; Le Bihan et al., 2001) and the resurgence, particularly in the neurosurgical community, of post mortem dissection according to the Klingler method (Türe, Yaşargil, Friedman, & Al-Mefty, 2000) has reignited an interest in the study of white matter anatomy. In the last decade, several fibre dissection studies have been reported, providing original anatomical data (Fernandez-Miranda et al., 2008; Martino, Vergani, Robles, & Duffau, 2010; Peltier et al., 2010) and elucidating the cortical termination of some controversial fascicles (Martino, Brogna, Robles, Vergani, & Duffau, 2010; Sarubbo, De Benedictis, Maldonado, Basso, & Duffau, 2011).

In this work, we present original data obtained according to the Klingler dissection of three neuroanatomically healthy

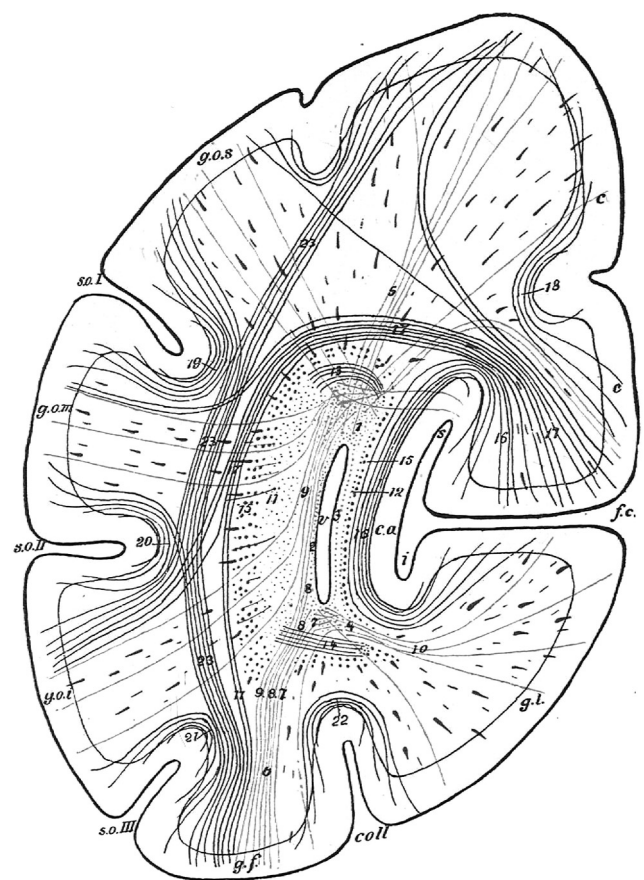


Fig. 1 – Schematic representation of the intralobar white matter fibres of the occipital lobe provided by Sachs (1892). Frontal section through a right hemisphere. v: ventricle (occipital horn); f.c.: fissura calcarina (calcarine fissure); coll.: collateral sulcus; s.o.I: sulcus occipitalis superior, s.o.II: sulcus occipitalis medium; s.o.III: sulcus occipitalis inferior; g.l.: gyrus lingualis; g.f.: gyrus fusiformis; g.o.s.: superior occipital gyrus; g.o.m.: middle occipital gyrus; g.o.i.: inferior occipital gyrus; 1–10: forceps; 11–14: stratum sagittale internum; 15: stratum sagittale externum; 16: stratum calcarinum; 17: stratum cunei transversum; 18: stratum proprium cunei; 19: stratum proprium s.o.I; 20: stratum proprium s.o.II; 21: stratum proprium s.o.III; 22: stratum proprium coll.; 23: stratum profundum convexitatis.

right hemispheres and compare these results with the original description of the occipital white matter provided by Sachs in 1892.

2. Materials and methods

Klingler dissection was performed on three right hemispheres obtained from the autopsy of two female and one male deceased human subjects, who did not suffer from any neurological and/or psychiatric disease. The specimens were collected at the Newcastle Brain Tissue Resource, Newcastle upon Tyne (UK). The Newcastle Brain Tissue Resource has ethical approval to use post mortem human specimens for research, and it complies with the Human Tissue Act. Specimens were fixed in 10% formalin solution for a minimum of three months. After removal of the pia–arachnoid membrane and cortical vessels, the hemispheres were frozen at -15°C for 15 days. The water crystallisation induced by the freezing process disrupts the structure of the grey matter (which has a high water content), thus making it easier to peel off the cortex from the brain surface. The freezing process also separates out the white matter fibres, facilitating the dissection of the fibre tracts. The specimens were washed under running water for several hours before performing the dissection.

The surface anatomy of each hemisphere was studied in detail, with identification of sulci and gyri. For the purpose of the current study, coronal cuts were performed at the same levels as originally described by Sachs (Fig. 2). Coronal slices were obtained, from anterior to posterior, at a plane passing through the splenium of the corpus callosum (cut 6), at a plane bisecting the precuneus and passing at the junction between the parieto-occipital sulcus and calcarine fissure (cut 4), at a plane bisecting the cuneus (cut 3) and at a plane dividing the posterior portion of the cuneus (cut 2). The white matter tracts identifiable in each coronal slice were identified and compared to the original dissections described by Sachs.

In addition, stepwise dissection of the white matter was also performed, from lateral to medial and from medial to lateral. To this aim, wooden spatulas were used to peel away the cortex. After removal of the cortical grey matter, the U-shaped fibres (also known as intergyral or short arcuate fibres) were identified. At this point the dissection was completed using blunt metallic dissectors with different tip sizes. Care was taken to separate the fibres using the blunt edge of the instrument, thus avoiding the generation of spurious tracts. Digital high-resolution photographs were taken throughout the procedure.

3. Results

3.1. Surface anatomy of the occipital lobe

On the medial surface of the hemisphere, two prominent sulci defining the anatomy of the occipital lobe were identified in all three hemispheres (Fig. 2a). The parieto-occipital sulcus was recognised at the boundary between the parietal and occipital lobe. This sulcus appeared to be uniformly uninterrupted (Ono, Kubik, & Abernathy, 1990) and separated the precuneus

(parietal lobe) from the cuneus (occipital lobe). The calcarine fissure, running from the occipital pole towards the splenium of the corpus callosum, divided the cuneus (superior to the fissure) from the lingual gyrus (inferior to the fissure). Both these sulci appeared to be rich in connecting gyri found in the deepest portion of the sulcus. Sachs compared these “gyri brevis” to the insular gyri found in the depth of the Sylvian fissure (Fig. 7a).

On the lateral surface of the brain, the occipital lobe appeared to be in continuity with the posterior part of the parietal and temporal lobes (Fig. 2b). On the dorsal edge of the lateral aspect, the outer margin of the parietoccipital sulcus was identified. Three horizontal sulci were observed on the convexity of the occipital lobe. The transverse occipital sulcus (called by Sachs *sulcus occipitalis superior*, s.o.I) lies lateral to the superior margin of the hemisphere and represents ideally the

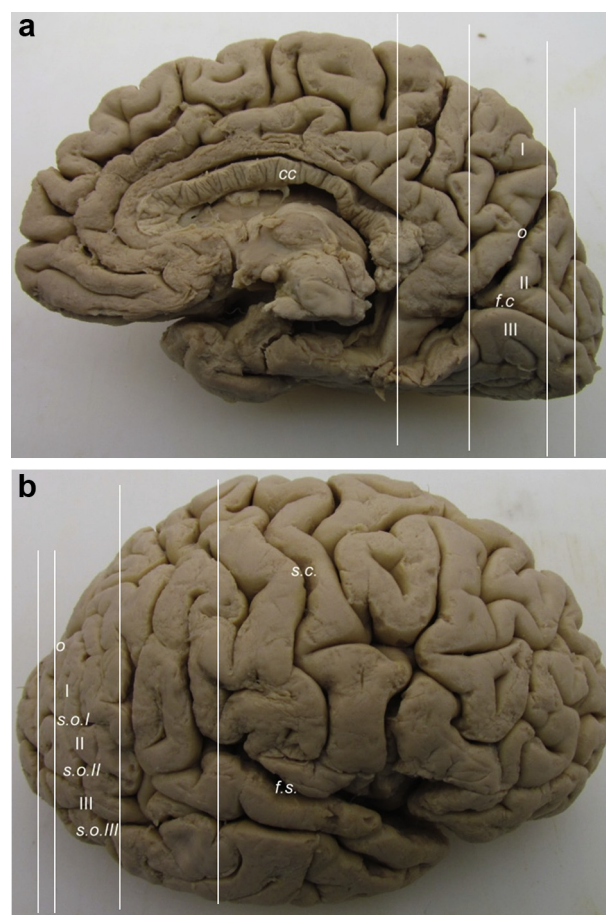


Fig. 2 – a (top panel): medial surface of a right cerebral hemisphere prepared according with Klingler technique. O: occipito-parietal fissure; f.c.: calcarine fissure; cc: corpus callosum; I: precuneus; II: cuneus; III: lingula. **b (bottom panel):** lateral surface of a right cerebral hemisphere prepared according with Klingler technique. O: occipito-parietal fissure; f.s.: sylvian fissure; s.o.I: sulcus occipitalis superior; s.o.II: sulcus occipitalis medium; s.o.III: sulcus occipitalis inferior. Vertical lines correspond to the coronal sections 6, 4, 3 and 2 (from anterior to posterior) as described by Sachs.

continuation – in the occipital lobe – of the intraparietal sulcus. The lateral occipital sulcus (*sulcus occipitalis medius*, s.o.II in Sachs' atlas) and the inferior occipital sulcus (*sulcus occipitalis inferior*, s.o.III) lie approximately at the level of the superior and inferior temporal sulci, respectively. These three horizontal sulci identify three gyri on the lateral surface of the occipital lobe: superior, middle and inferior. It has to be noted (as was also recognised by Sachs himself) that the lateral aspect of the occipital lobe shows a great variability in the pattern and presentation of sulci and gyri, with in particular the lateral and inferior sulci being less consistent (“usually interrupted and branched and therefore less clear”). The three gyral pattern described here was recognised in two of our three specimens, while in one specimen only two lateral gyri were found (the inferior sulcus not being present). This variability has been also reported for larger cohorts in contemporary studies (Alves, Ribas, Párraga, & de Oliveira, 2012; Iaria & Petrides, 2007; Iaria, Robbins, & Petrides, 2008).

On the basal surface of the brain, the occipital lobe continued with the temporal lobe. The more prominent sulcus at this level was the collateral sulcus that divided the lingual gyrus (medially) from the fusiform gyrus (laterally). More laterally, the occipito-temporal sulcus divided the fusiform gyrus (medially) from the inferior temporal gyrus.

3.2. Short association fibres

After removal of the cortex, short U-shaped fibres interconnecting neighbouring gyri were encountered first.

On the medial surface of the brain, dissection of the parietooccipital sulcus showed the presence of U-fibres running in the depth of the sulcus and connecting the

superior margin of the cuneus with the inferior aspect of the precuneus (Fig. 7b). Dissection of the calcarine fissure demonstrated a large and relatively thick bundle of U-shaped fibres connecting the upper and lower edges of the calcarine cortex (Fig. 7c). Coronal sections performed just posterior to the junction between the parietooccipital sulcus and the calcarine fissure also clearly demonstrated U-shaped fibres running between the upper and lower bank of the calcarine fissure (Figs. 4–6). These fibres correspond to the *stratum calcarinum* as described by Sachs. As previously mentioned, both the parietooccipital sulcus and the calcarine fissure demonstrate a rich pattern of connecting gyri. Klingler dissection showed that the location of the U-shaped fibres lying in the depth of these two sulci correspond with the surface anatomy of the connecting circonvolutions. It has to be noted with great interest that the dissection of the medial aspect of the calcarine fissure also demonstrated a bundle of fibres previously not described by Sachs or any other post mortem dissection work the authors are aware of. This tract runs deep within the calcarine fissure from the inferior portion of the cuneus to the superior and anterior aspect of the lingual gyrus (where the lingual gyrus blends with the parahippocampal gyrus). The bundle presents with a superior-inferior and anterior curvature. Owing to its peculiar shape, this novel pathway has been named “sledge runner” fasciculus (Catani et al., 2014).

After removing the cortex of the cuneus, U-shaped fibres running between the superior and inferior portion of the cuneus (i.e., to the superior aspect of the calcarine fissure) were exposed (Fig. 7d). These fibres were replicated on coronal sections (Fig. 5). This bundle of fibres corresponds to the *stratum proprium cunei* (layer of the cuneus) of Sachs.

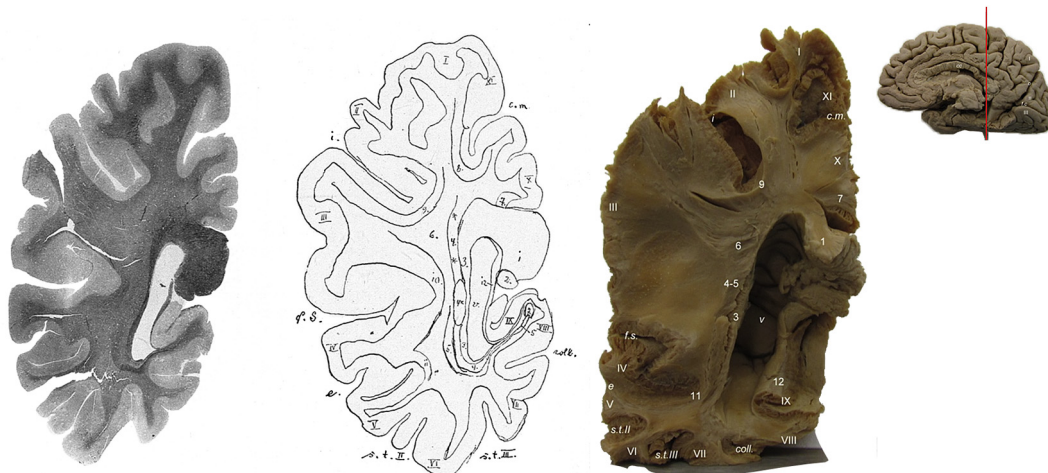


Fig. 3 – Comparison between Sachs's histological atlas and blunt dissections on coronal section through the splenium of the corpus callosum (upper right inset). Histological slice corresponding to section 6 of Sachs (left); schematic diagram of the fibres visualized in photo 6 (middle); coronal cut of a right hemisphere prepared with Klingler's method (right). I: posterior central gyrus; II: superior parietal lobule; III: marginal gyrus; IV: superior temporal gyrus; V: middle temporal gyrus; VI: inferior temporal gyrus; VII: fusiform gyrus; VIII: hippocampal gyrus; IX: Ammon gyrus; X: gyrus fornicatus; XI: paracentral lobule. v: ventricle; i: interparietal fissure; f.s.: sylvian fissure; s.t.II: middle temporal sulcus; s.t.III: inferior temporal sulcus; e: sulcus parallelis; coll: collateral sulcus; c.m.: calloso-marginal sulcus; 1: splenium of corpus callosum; 2: inferior portion of forceps; 3: tapetum; 4: stratum sagittale internum; 5: stratum sagittale externum; 6: arcuate fasciculus; 7: cingulum; 8: stratum proprium of the calloso-marginal sulcus; 9: stratum proprium of interparietal sulcus; 10: vertical layer of the convexity; 11: stratum proprium sulcus parallelis. 12: column of the fornix.

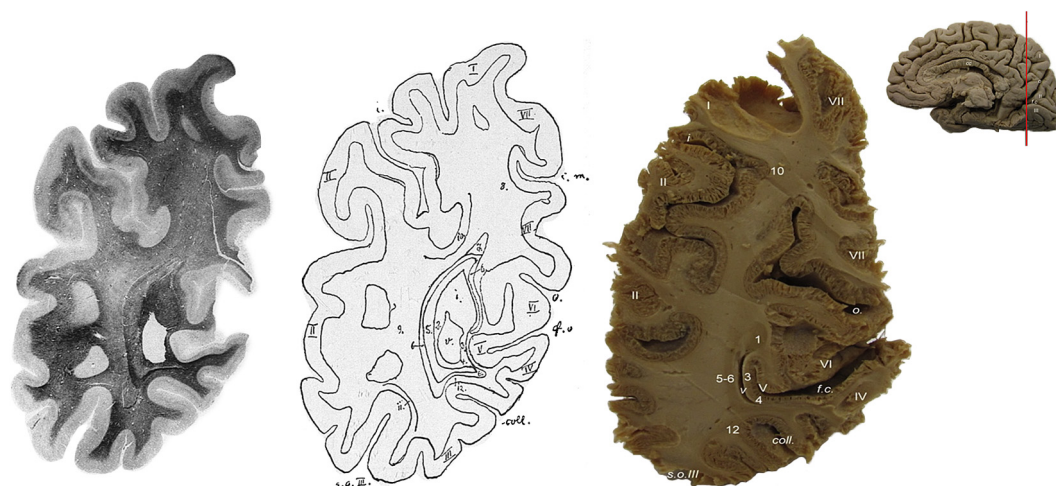


Fig. 4 – Coronal section through the precuneus and anterior edge of the calcarine fissure (upper right inset). Histological slice corresponding to section 4 of Sachs (left); schematic diagram of the fibres visualized in photo 4 (middle); coronal cut of a right-sided hemisphere prepared according to Klingler's method (right) I: superior parietal lobule; II: angular gyrus; III: fusiform gyrus; IV: lingual gyrus; V: calcar avis; VI: cuneus; VII: precuneus; v: ventricle (occipital horn); i: interparietal fissure; s.o.III: inferior occipital sulcus; c.m.: calloso-marginal fissure; o: occipito-parietal fissure; f.c.: calcarine fissure; coll: collateral sulcus; 1–4: fibres of forceps major; 5: stratum sagittale internum; 6: stratum sagittale externum; 7: stratum transversum cunei (transverse layer of the cuneus); 8: stratum proprium of precuneus; 9: vertical layer of the convexity; 10: stratum proprium of interparietal fissure; 11: stratum proprium of inferior occipital sulcus; 12: stratum proprium collateral sulcus.

On the coronal sections, a bundle of fibres originating at the level of the cuneus and running inferiorly and laterally, was also observed (Figs. 5 and 6). This tract was directed towards the lateral wall of the occipital horn, coursing lateral to the stratum sagittale (see below) before pointing more laterally, towards the convexity of the occipital lobe. This bundle

corresponds to the *stratum transversum cunei* (transverse layer of the cuneus) described by Sachs. It was unfortunately not possible to trace the cortical terminations of this bundle, as it was difficult to differentiate its fibres from the outer layer of the stratum sagittale medially and from the fibres of the convexity laterally.

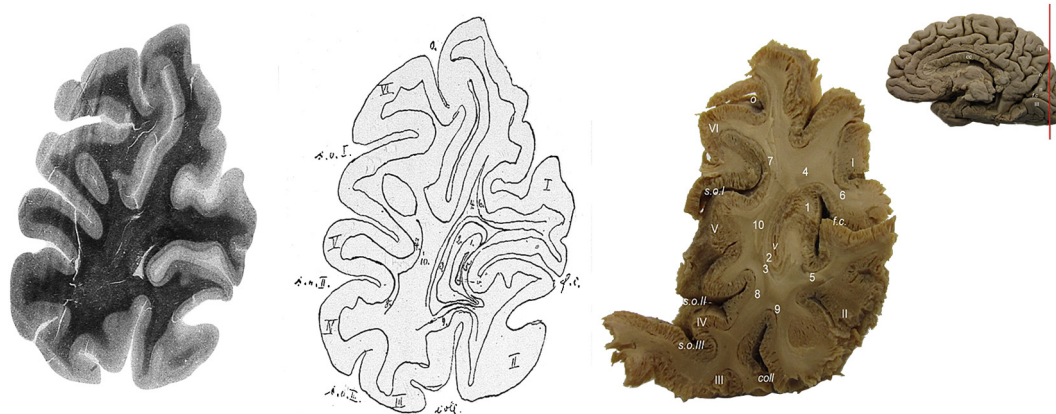


Fig. 5 – Coronal section through the middle portion of the cuneus (upper right inset). Histological slice corresponding to section 3 of Sachs (left); schematic diagram of the fibres visualized in photo 3 (middle); coronal cut of a right-sided hemisphere prepared according to Klingler's method (right) I: cuneus; II: lingual gyrus; III: fusiform gyrus; IV: inferior occipital gyrus; V: middle occipital gyrus; VI: superior occipital gyrus; v: ventricle (occipital horn); f.c.: calcarine fissure; coll: collateral sulcus; s.o.I: superior occipital sulcus; s.o.II: middle occipital sulcus; s.o.III: inferior occipital sulcus; o: occipito-parietal sulcus; 1: forceps; 2: stratum sagittale internum; 3: stratum sagittale externum; 4: transverse layer of the cuneus; 5: stratum calcarinum; 6: stratum proprium of cuneus; 7: stratum proprium of s.o.I; 8: stratum proprium of s.o.II; 9: stratum proprium of collateral sulcus; 10: deep layer of the convexity.

Dissection of the lateral surface of the occipital lobe demonstrated a continuous system of U-shaped fibres connecting neighbouring gyri. Thin U-shaped fibres were observed running deep within the cortex of the superior, middle and inferior occipital sulci (Figs. 4–6 and 8a). Sachs named these fibres collectively as “*stratum verticale convexitatis*” (vertical layer of the convexity), naming each individual fibre after the sulcus containing it (e.g., *stratum proprium sulci occipitalis I*, and so on). Deeper to this superficial layer of U-shaped fibres, a vertical bundle running from the superior to the inferior region of the occipital lobe was encountered (Figs. 5 and 8a). This tract, rather thin and running lateral to the *stratum sagittale*, possibly corresponds to the *stratum profundum convexitatis* of Sachs (deep layer of the convexity). In our dissections it was difficult to follow this bundle for its entire course, as it appeared to merge with the U-shaped fibres of the convexity.

At the basal surface of the occipital lobe, a well-defined U-shaped fibre tract, running deep in the collateral sulcus and connecting the fusiform gyrus with the lingual gyrus was demonstrated (Figs. 4–6). This corresponds to the *stratum proprium sulci collateralis* (layer of the collateral sulcus) identified by Sachs.

3.3. Stratum sagittale

A dense bundle of fibres with a sagittal trajectory and extending between the temporal and the occipital lobes was clearly demonstrated in all coronal sections. This represented the *stratum sagittale* described by Sachs (Figs. 4–6). Fibres of the *stratum sagittale* are contained between the white matter of the convexity and the ventricle. They extend in a C-shaped layer embracing the superior, lateral and

inferior walls of the occipital horn of the ventricle; the portion encasing the floor of the ventricle is more pronounced than the portion overlying the roof. The *stratum sagittale* appears to be larger at the level of the temporal lobe, decreasing progressively in size as it approaches the occipital pole.

Continuing the dissection of the white matter from lateral to medial, two layers of the *stratum sagittale* were identified; these two layers were named *stratum sagittale internum* (internal layer) and *stratum sagittale externum* (external layer) by Sachs (Fig. 8b). The two layers are contiguous and they are more difficult to appreciate on coronal sections, and in most of our cuts they were not readily identifiable as distinct layers. The two layers can be better appreciated after carefully separating the *stratum externum* from the *stratum internum* with fine blunt dissectors at the level of the lateral and superior wall of the occipital horn. A distinction between the two layers at the level of the floor of the ventricle was not possible in our study.

3.4. Callosal fibres

On coronal sections, fibres surrounding the dorsal, lateral and medial portion of the occipital horn of the ventricle were also identified. These fibres were situated medially to the above described *stratum sagittale*, representing the layer of white matter directly in contact with the walls of the ventricle. These fibres correspond to the forceps major, the radiation of commissural fibres arising from the splenium of the corpus callosum and connecting the occipital cortices. Forceps fibres were mostly evident at the level of the splenium of the corpus callosum (Fig. 3). After leaving the splenium, these fibres follow an inferior trajectory laterally to the lateral wall of the

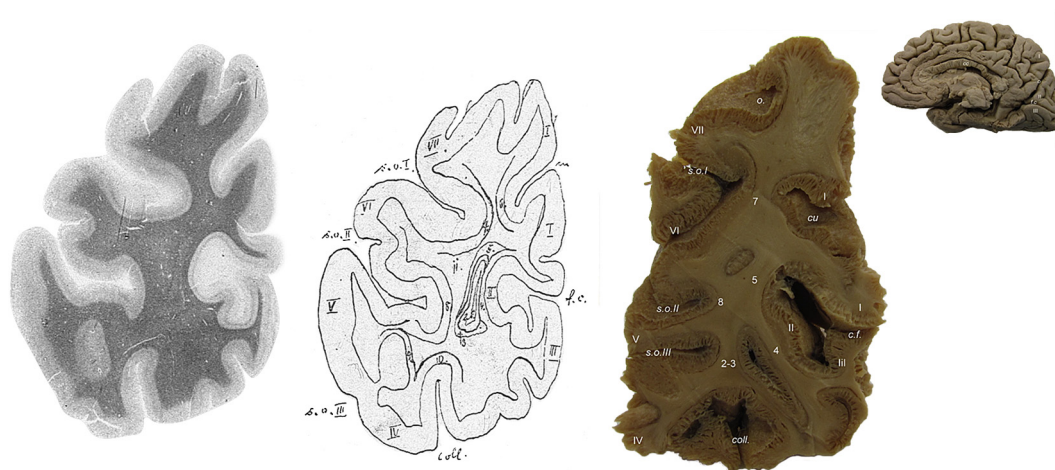


Fig. 6 – Coronal section through the posterior portion of the cuneus (upper right inset). Histological slice corresponding to section 2 of Sachs (left); schematic diagram of the fibres visualized in photo 2 (middle); coronal cut of a right-sided hemisphere prepared according to Klingler’s method I: cuneus; II: calcar avis; III: lingual gyrus; IV: fusiform gyrus; V: inferior occipital gyrus; VI: middle occipital gyrus; VII: superior occipital gyrus; s.o.I: superior occipital sulcus; s.o.II: middle occipital sulcus; s.o.III: inferior occipital sulcus; f.c.: calcarine fissure; coll.: collateral sulcus; cu: sulcus of cuneus; 1: forceps; 2: *stratum sagittale internum*; 3: *stratum sagittale externum*; 4: *stratum calcarinum*; 5: transverse layer of the cuneus; 6: *stratum proprium* of the cuneus; 7: *stratum proprium* of s.o.I; 8: *stratum proprium* of s.o.II; 9: *stratum proprium* of s.o.III; 10: *stratum proprium* collateral sulcus; 11: deep layer of the convexity.

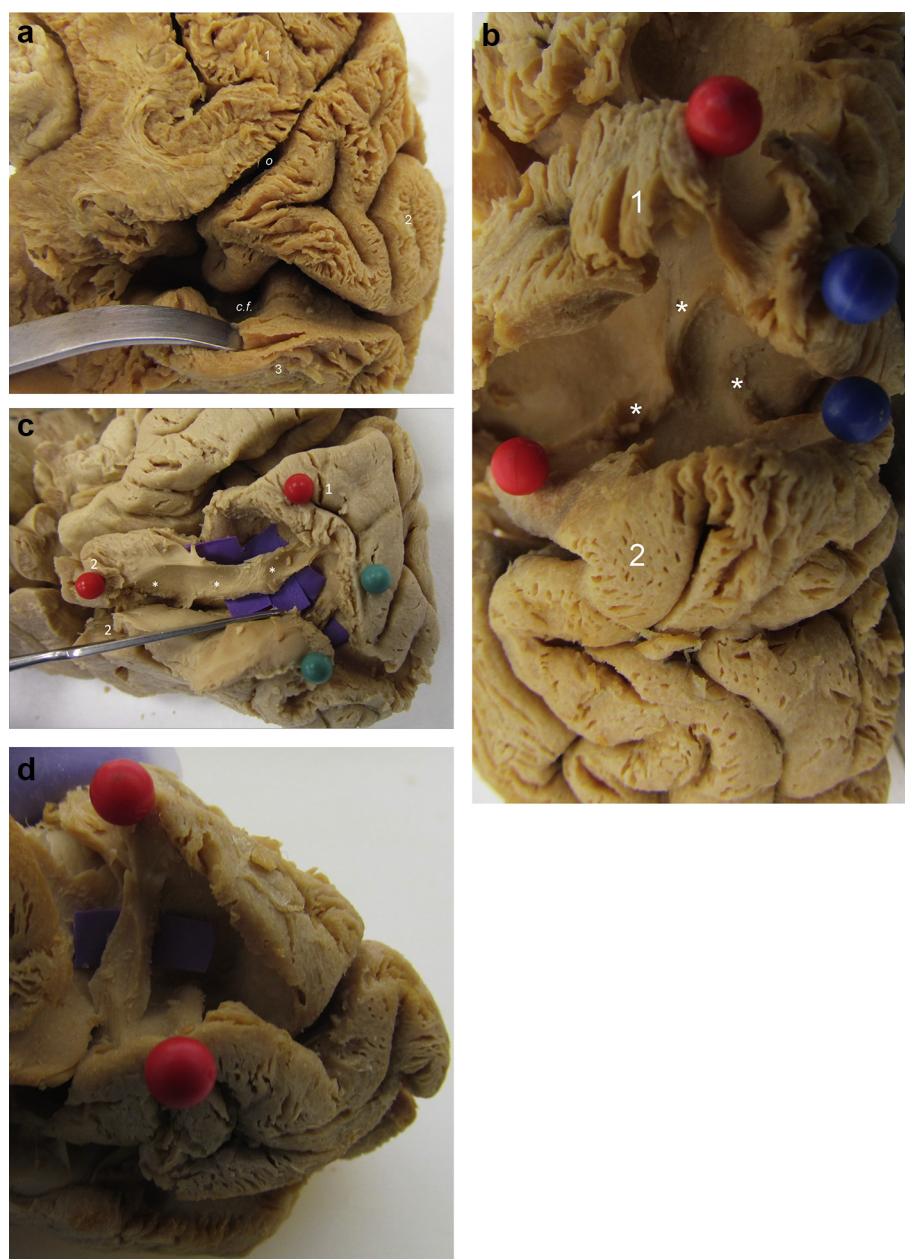


Fig. 7 – Dissection of the medial sulci of the occipital lobe (right hemisphere, sagittal plane). a) Medial surface of the brain prior to dissection. 1: precuneus; 2: cuneus; 3: lingual gyrus. Note the rich pattern of gyri in the calcarine fissure. b) Dissection of the parietoccipital sulcus, showing U-shaped fibres (*) laying in the depth of the sulcus and connecting the precuneus (1) with the cuneus (2). Red and blue pins represent the cortical terminations of the fibres. c) Dissection of the calcarine fissure, showing the “sledge runner” fascicle (") running from the cuneus (1) to the antero-superior portion of the lingula (2). Red pins indicate the cortical termination of the tract. U-shaped fibres laying in the depth of the sulcus and connecting the upper and lower edges of the calcarine fissure are also demonstrated (green pins). These fibres correspond to the stratum calcarinum of Sachs. Small pieces of purple paper have been inserted to show the complete dissection and continuity of the fibres. d) dissection of the cuneus, showing U-fibres connecting the upper (1) and lower (2) part of the cuneus. These fibres correspond to the *stratum proprium cunei* of Sachs. Red pins indicate the cortical termination of the fibres, while the small pieces of purple paper have been inserted to show the complete dissection and continuity of the fibres.

ventricle and will eventually terminate within the temporal lobe. This branch of the posterior callosal fibres is referred to as *tapetum*. More posteriorly, as the occipital horn of the ventricle decreases in size, callosal fibres appeared less well

differentiated from the surrounding white matter. At this level, they were nonetheless recognisable, particularly at the superior and medial portion of the ventricular walls (Figs. 4 and 5).

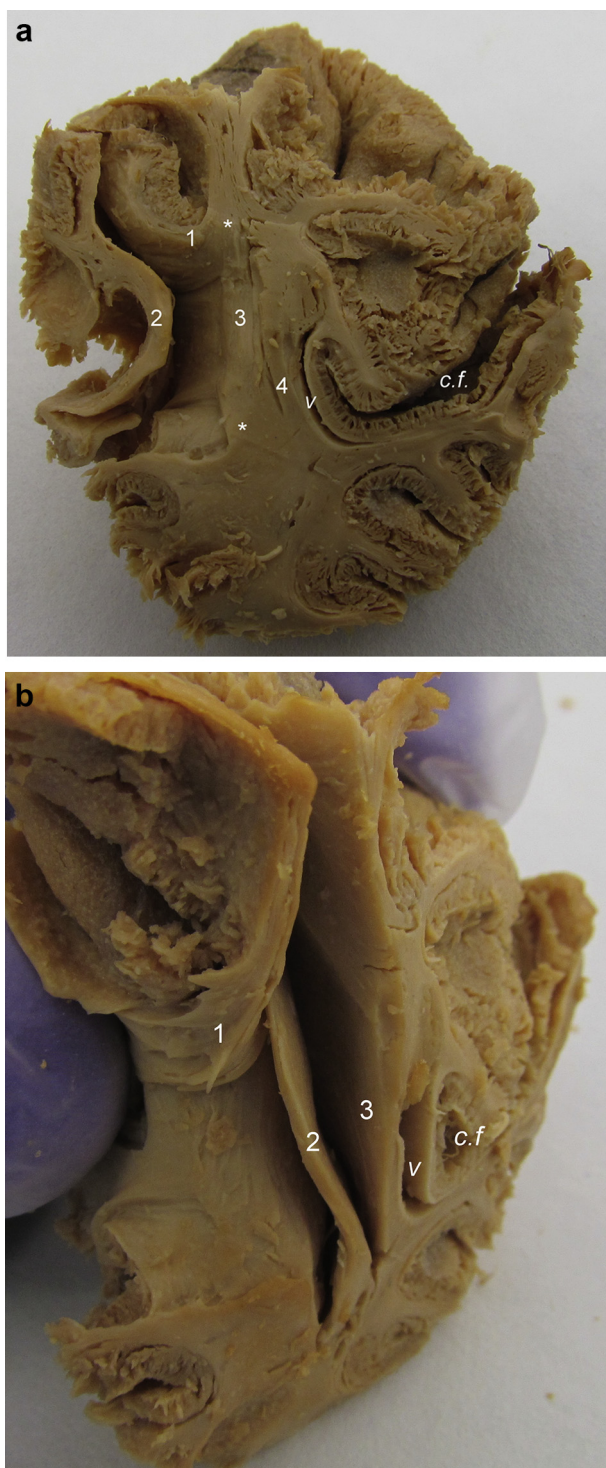


Fig. 8 – Lateral to medial dissection of the occipital lobe (right hemisphere, coronal plane). a) Removal of the cortex overlying the sulci at the convexity revealed a system of U-shaped fibres connecting neighbouring gyri (1 and 2). These fibres correspond to the stratum proprium sulci occipitalis according to Sachs. Deeper to these U-shaped fibres, a vertical bundle was identified (3), corresponding to the stratum profundum convexitatis described by Sachs. This bundle, lateral to the stratum sagittale (4) appeared to blend with the superficial fibres of the convexity (*). V: ventricle, c.f.: calcarine fissure. b) Dissection more medial

4. Discussion

4.1. The anatomy of intralobar occipital fibres

The atlas of Sachs is one of the most accurate descriptions of the occipital fibres in humans, and it still represents a most valuable basis of comparison for those who want to approach this subject.

In the present study we investigated the intralobar white matter fibres of the occipital lobes preparing three hemispheres according to the Klingler's method. We performed coronal sections of the hemispheres at the same levels described in the historical atlas by Sachs, in order to compare our findings with the original descriptions. Overall, a striking concordance was observed between the two techniques (histology in Sachs's work and dissections in the present work). All major tracts described by Sachs were also clearly identified in our three specimens. The small differences observed in some of the coronal sections (where not every single fibre could be identified with the two methods) can be explained with the law of variability expressed by Sachs in the Introduction to his atlas: "There are no two brains that are identical in all their details. This variability is also observed in the arrangement and development of the white matter. The cortex and the white matter fibres are mutually dependent on each other. If a particular area of cortex is under-developed in a brain, then there is also a paucity of fibres originating from this area." (see Forkel et al., 2014). It is therefore possible that small differences exist, particularly at the level of short arcuate fibres. This may also be reflected by the fact that a coronal cut performed at the same level can show a certain amount of fibres in one specimen and other fibres in another.

One fascicle, although clearly visualized, was particularly difficult to follow with the Klingler's technique. This is the "transverse stratum of the cuneus", running between the cuneus in a medial to lateral direction, curving lateral to the stratum sagittale and eventually pointing towards the convexity. Particular uncertainty persists regarding its cortical terminations, which could not be determined with the dissection method. The main difficulty encountered was to separate this bundle from the stratum sagittale medially and from the fibres of the convexity laterally. At this level in particular the dissection was rendered difficult due to fibre crossing between the "transverse stratum of the cuneus", that shows an horizontal direction, and the "stratum proprium convexitatis", that has a vertical course instead. This problem was also encountered by Sachs who had to conclude the description of the bundle with a note of uncertainty: "These fibres could thus far not be traced [...] along their trajectory through the stratum proprium convexitatis towards the cortex. They potentially reach the cortex of the whole convex part and part of the inferior surface of the occipital lobe and thus form the association

than a) within the same hemisphere. The vertical stratum profundum convexitatis has been partially removed (1) to expose the external (2) and internal (3) layers of the stratum sagittale at the level of the lateral wall of the ventricle (v). Note the longitudinal course of the fibres of the stratum sagittale.

pathway between the cuneus and the convexity” (see Forkel et al., 2014). Fibre crossing is a problem common to most, if not all, the different techniques applied to the study of white matter anatomy. The recent development of advanced Magnetic Resonance (MR)-based tractography algorithms, such as spherical deconvolution (that is able to partially overcome the limitation of crossing fibres by identifying multiple fibre orientations within a single brain voxel) may in part overcome this problem (Dell’Acqua et al., 2007, 2010). A limitation of the current study, which is not infrequent to post mortem studies, is the small number of specimens available for dissection. It is possible that with more specimens, a clearer delineation of the “transverse stratum of the cuneus” might be possible. This may be addressed in future studies.

Several differences between the present work and the atlas of Sachs were observed and shall be discussed in detail. The first dissimilarity regards the “stratum profundum convexitatis” (deep stratum of the convexity), which was considered by Sachs as a separate white matter bundle. Although identified as a contingent of vertical fibres running from the superior aspect of the occipital lobe to its inferior surface, this layer of white matter did not appear – with Klingler dissections – to be clearly separated from the superficial U-shaped fibres of the convexity. It is therefore questionable if this “stratum profundum convexitatis” should be considered as an independent white matter tract. Schmahmann and Pandya, in their axonal tracing work on the monkey brain, were also able to identify fibres that link the dorsal and ventral aspects of the occipital lobe, but considered them as being part of a vertical component of the Inferior Longitudinal Fasciculus (ILF) (Schmahmann and Pandya, 2009).

The second discrepancy was found at the level of the stratum sagittale. According to Sachs, the stratum sagittale is divided in an external layer, that conveys corticopetal fibres from the thalamus to the occipital lobe, and an internal segment that conveys corticofugal fibres from the cortex to the thalamus. In Sachs’s view, the “stratum sagittale internum” is therefore a projection system, while the “stratum sagittale externum” is an association system, connecting the occipital lobe exclusively with the temporal lobe: “*The occipital lobe has only one long association tract, namely the stratum sagittale externum, that connects to the temporal lobe*” (see Forkel et al., 2014). In our dissections, it was possible to differentiate between the two layers only on the superior and lateral aspect of the ventricular wall. At the level of the floor of the ventricle a thick bundle of white matter, deep in the collateral sulcus, was encountered, with no clear distinction between an external and internal layer. Recent studies, using post mortem dissections and in vivo diffusion tensor imaging tractography, have demonstrated the presence of a long association fascicle that runs along the superior and lateral portion of the ventricular wall, lateral to the optic radiations, before reaching its cortical terminations. This bundle, that connects the frontal to the occipital lobe, corresponds to the inferior fronto-occipital fasciculus (IFOF), originally described by Burdach and later by Curran (Burdach, 1822; Catani, Howard, Pajevic, & Jones, 2002; Curran, 1909; Fernandez-Miranda et al., 2008; Forkel et al., 2012; Martino, Brogna, et al., 2010). The white matter encasing the inferior wall and the floor of the ventricle could correspond to the ILF, a bundle connecting the temporal and

the occipital lobe, and running inferolaterally to the optic radiations and the ventricles (Catani, Jones, Donato, & Ffytche, 2003; Fernandez-Miranda et al., 2008).

A final relevant difference was observed in the dissection of the medial aspect of the calcarine fissure. In agreement with Sachs, U-shaped fibres connecting the superior and inferior edges of the fissure were clearly demonstrated in our dissections. However, in contrast to Sachs, we identified an additional fascicle running anteriorly from the inferior portion of the cuneus to the supero-anterior portion of the lingual gyrus. This fascicle, due to its peculiar shape, has been named the “sledge runner” (Catani et al., 2014). It is likely that Sachs missed this fascicle as he only examined coronal sections. The “sledge runner” is a thin fascicle, with a postero-anterior course in the sagittal plane, and can therefore be either lost or confused with the other U-shaped fibres running in the calcarine fissure in the coronal plane. Our findings represent the first demonstration of this fascicle in post mortem human specimens, whilst confirming its discovery in tractography studies (Catani et al., 2014).

4.2. The functional and connectional anatomy of the occipital lobe

The study of the occipital lobe anatomy has seen an evolution across the centuries. While early studies (Bolton, 1900; Retzius, 1896; Sachs, 1892) provided a gross anatomical description of the cortical and subcortical anatomy, following reports focused on the cytoarchitecture of the occipital cortex, identifying the existence of distinct cortical areas based on a different neuronal organisation of the six cortical layers (Amunts, Malikovic, Mohlberg, Schormann, & Zilles, 2000; Brodmann, 1909; Von Economo & Koskinas, 1925). These studies established that the primary visual cortex, organised around the calcarine fissure (Brodmann area 17) is surrounded by a cytoarchitecturally different ring of cortex (Brodmann area 18) which is in turn surrounded by an even larger area, corresponding to much of the remaining occipital cortex (Brodmann area 19). A further step was obtained thanks to neurophysiological investigations performed in the nonhuman primate, where direct electrical recording was employed to map the receptive fields of individual neurons after visual stimuli (Kaas, 2004; Sereno & Tootell, 2005). Several visual areas were thus identified, extending beyond the primary visual cortex (referred to as area V1) and even extending beyond the classic boundaries of the occipital lobe, with “visual” areas observed in the temporal and parietal lobes (for a review see Felleman & Van Essen, 1991; Vezoli et al., 2004). The existence of different visual areas has been confirmed in the human brain using non-invasive techniques, such as functional MRI or magnetoencephalography (Anderson, Holliday, Singh, & Harding, 1996; Dougherty et al., 2003; Dupont, Orban, De Bruyn, Verbruggen, & Mortelmans, 1994; Sack, Kohler, Linden, Goebel, & Muckli, 2006; Stiers, Peeters, Lagae, Van Hecke, & Sunaert, 2006; Tootell & Hadjikhani, 2001; Zeki et al., 1991). These studies led to the generation of “functional” maps of the occipital lobe (Van Essen, 2003).

The study of brain connections is the important anatomical correlate for an understanding of function (Mesulam,

2005). In the monkey brain, connections of the occipital lobe have been investigated in great detail, with an emphasis on the intralobar networks. In particular, it has been demonstrated in animal studies that only a minority of stellate cells in layer 4 receive their input from the lateral geniculate nucleus (LGN), while about 80% of the synapses in the occipital lobe receive an input from adjacent occipital areas (Binzegger, Douglas, & Martin, 2004). Such microcircuits may play an important role in the reinforcement of the input from the LGN (Sincich & Horton, 2005). On the basis of these connection studies, it has been possible to delineate “hierarchical” maps of the occipital lobe connections (Felleman & Van Essen, 1991). In the human brain, less attention has been paid to the intralobar connectivity of the occipital lobe (Thiebaut de Schotten, Urbanski, Valabregue, Bayle, & Volle E, 2012). Previous studies employing post mortem Klingler dissection and in vivo tractography focused on the long association tracts connecting the occipital to the frontal lobe via the IFOF (Catani et al., 2002; Fernandez-Miranda et al., 2008; Forkel et al., 2012; Martino, Brogna, et al., 2010; Sarubbo et al., 2011), or to the temporal lobe via the ILF (Catani et al., 2003; Fernandez-Miranda et al., 2008). The optic radiations, with their relationship to the ventricles and their cortical termination at the level of the calcarine fissure have also been studied in detail (Kucukyuruk, Richardson, Wen, Fernandez-Miranda, & Rhoton, 2012; Mahaney & Abdulrauf, 2008; Yogarajah et al., 2009). The anatomy of short association fibres, such as the superficial U-shaped fibres of the convexity, the *stratum calcarinum*, and the “sledge runner” tract have not been described in previous tractography studies (Catani et al., 2014). In the present work, we updated the original work performed by Sachs, describing the detail of intralobar fibres with post mortem dissections.

The putative role of some of the fibres described can be suggested on the basis of the aforementioned functional studies. The *stratum calcarinum*, with U-shaped fibres connecting the superior and the inferior edges of the calcarine fissure, may play a role in the integration of visual information from the upper and inferior portions of the visual fields. The U-shaped fibres of the superficial layer of the convexity (corresponding to the *stratum superficialis* and *stratum profundus convexitatis* of Sachs), connect the cortical area between the lateral occipital and the inferior occipital sulci (corresponding to functional area middle temporal, MT) with the neighbouring gyri, including area V3A. The area MT has been associated with detection of visual stimuli (Bartels, Logothetis, & Moutoussis, 2008). Damage to the network that is subserved by these superficial fibres might clinically manifest as akinetopsia, the inability to detect moving objects (Thiebaut de Schotten et al., 2012). More anteriorly, at the junction between the temporal and occipital lobe, the system of superficial U-shaped fibres and vertical fibres of the *stratum profundus convexitatis* represent a connection between ventral occipito-temporal regions and superior occipital and parietal regions. These fibres, considered together in the present study, may correspond to the vertical occipital fasciculus (VOF) of Wernicke. A recent study by Yeatman, Rauschecker, and Wandell (2013) has suggested that the VOF has an important role in connecting the Visual Word Form Area (VWFA), a part of the ventral occipito-temporal cortex

specialised in the visual formation of words and reading (Dehaene, Le Clec, Poline, Le Bihan, & Cohen, 2002; Wandell, Rauschecker, & Yeatman, 2012), with language areas including the angular gyrus (Yeatman et al., 2013). Damage to the white matter surrounding the VWFA has been related to the development of pure alexia (Epelbaum et al., 2008; Greenblatt, 1973). The “sledge runner” tract, connecting the superior edge of the calcarine fissure and the anterior portion of the lingual gyrus (where the occipital lobe and the parahippocampal gyrus blends together) can be seen as a tract for the integration of visual stimuli from the inferior portion of the visual field and parahippocampal areas involved in the recognition of places (Arcaro, McMains, & Kastner, 2009; Catani et al., 2014). Finally, the clinical importance of the *stratum sagittale* of the occipital lobe has been recently emphasised by Viegas, Moritz-Gasser, Rigau, and Duffau (2011). In this retrospective review of patients operated for World Health Organisation (WHO) grade II gliomas encroaching on the occipital lobes, semantic paraphasias were induced after intraoperative stimulation of the IFOF (for lesions located in the language dominant hemisphere). The IFOF therefore constituted the antero-lateral margin of resection, preserving language function in patients undergoing an occipital lobectomy.

5. Conclusions

The human occipital lobe presents a rich and complex network of intralobar fibres, arranged around the ventricular wall. The general principle whereby short association fibres appear to be more superficial, whilst long ranging association fibres are located further away from the cortex and are in contact with the walls of the ventricle, can be confirmed in this study. Overall, good concordance was observed between our Klingler dissection in three right hemisphere specimens and the histological descriptions provided by Sachs’s atlas. The most important difference between both studies, is the description of a novel occipital pathway, namely the “sledge runner” connecting the cuneus to the lingual gyrus, described for the first time in a post mortem study. A comprehensive translation of the original work is enclosed to this research work for facilitated comparison and future reference.

Conflict of interest

The authors declare no financial or personal conflict of interest related to the present work.

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Special issue: Historical paper

The white matter of the human cerebrum: Part I The occipital lobe by Heinrich Sachs



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ABSTRACT

This is the first complete translation of Heinrich Sachs' outstanding white matter atlas dedicated to the occipital lobe. This work is accompanied by a prologue by Prof Carl Wernicke who for many years was Sachs' mentor in Breslau and enthusiastically supported his work.

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1. Introduction

Born in 1863, Heinrich Sachs was a German neurologist and neuroanatomist who obtained his specialisation in neurology

and psychiatry with Carl Wernicke in Breslau (Forkel, 2014). Sachs published on amyotrophic lateral sclerosis (1885), aphasia (1893; 1905), and traumatic neurosis (1909), but arguably his most distinctive contribution was in the field of white matter neuroanatomy. Whilst still a doctor in training he

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spent most of his time looking at series of cross-sections obtained from human brains. This painstaking effort resulted in the publication of the first atlas of the occipital lobe connections in the human brain (Sachs, 1892). Sachs's atlas contains detailed descriptions of the methodological approaches he employed, which makes the text not always an easy reading; but the figures are beautifully informative and include many previously undescribed tracts. For example, Sachs defined three different layers in the deep white matter of the occipital lobes, which he correctly identified as fibres of the splenium (forceps corporis callosi, see page 15), optic radiations (stratum sagittalis internum, see page 18) and the association pathways (stratum sagittalis externum, see page 20). These were later indicated with the name *stratum sagittalis* of Sachs in recognition of his work. He also introduced a new nomenclature for the vast number of U-shaped fibres running near the cortical surface of the occipital cortex. The knowledge of these tracts had direct clinical relevance as differences between apperceptive and associative visual agnosia could be explained in terms of primary visual cortex damage and damage to associative U-shaped fibres, respectively (Lissauer, 1890). In contemporary neuroscience we have understood that within the occipital lobe these U-shaped fibres mediate crosstalk between the ventral visual stream dedicated to objects-perception (the 'what' pathway) and the dorsal visual stream dedicated to place location and motion perception (the 'where' pathway).

Sachs' mentor Wernicke was an enthusiastic advocate of his anatomical insights and encouraged his trainee to further pursue this research. The atlas was in fact intended to be a multi-volume project in which subsequent books would have been dedicated to the function and clinical correlates of each tract. This was an ambitious project in the footsteps of the great clinical anatomists of the time. Unfortunately, Sachs did not complete what he had set out to accomplish and never returned to his master plan in the four decades he continued working as physician at the neurology and psychiatry clinic in Breslau.

Despite its importance, Sachs's atlas went unnoticed for decades. This is in part due to the availability of more detailed information on connectional anatomy derived from axonal tracing studies performed in animals. Also the lack of an integral translation from German to English did not facilitate its dissemination.

We believe that with the advent of novel MRI-based methods to study connections in the human brain, the work of Sachs could be of great relevance to contemporary neuroscience. This is particularly true for those tracts that may underlie uniquely human abilities. The vertical fasciculus of Wernicke, for example, connects relevant areas for reading. Sachs describes this tract in detail and credits his description to Wernicke (see page 26). Despite this tract being one of the largest intraoccipital connections, its function has remained unknown. More recent studies in patients with lesions to this white matter tract or its cortical projections suggest that it may have a role in reading (Yeatman, Rauschecker, & Wandell, 2013). Other tracts described by Sachs are still waiting to be ascribed a specific functional correlate.

Sachs's occipital tracts have been recently replicated using post mortem Klinger dissection (Vergani, Mahmood, Morris,

Mitchell, & Forkel, 2014). Detailed tractography studies are needed to characterise the in vivo anatomy of these tracts in terms of interindividual variability as previously shown for tracts of other lobes (Catani et al., 2007; 2012; Forkel et al., 2014; Lopez-Barroso et al., 2013).

2. Translation

In Memoriam to Dr. Heinrich Lissauer, Assistant at the psychiatric clinic in Breslau. Publications from the psychiatric clinic in Breslau.

"The white matter of the human cerebrum. Part 1. The Occipital Lobe" by Dr. med. Heinrich Sachs, neurologist in Breslau with a prologue by the medical officer of health Prof. Dr. C. Wernicke, including 3 figures and 8 plates.

3. Prologue by Wernicke

The present work is the first contribution to a series of publications dedicated to the investigation of the brain and its functions in health and pathology. This field of research is still heavily under investigated and nearly every contribution to it is a step forward similar to an expedition into unknown territory comparable to the "deepest Africa". The integration of clinical observations and anatomical aspects has constantly proven to be a reliable method to move forward. The advances in anatomy, which are naturally slow, will be followed promptly by our clinical experience.

The anatomy of the white matter of the cerebrum always intrigued me as the link between all delicate clinical methods; hence, I appreciate with great satisfaction that our colleague Sachs made such an encouraging start with the present work, which is of the highest standard in terms of its content and structure. May future publications be equally well received by colleagues. Breslau, January 1892.

4. Sachs' atlas

This work can be considered as the first part of a more extensive work on the white matter fibre trajectory in the healthy adult human brain. The dissections presented here were obtained in the psychiatric clinic in Breslau. I shall take the liberty to express my gratitude towards Professor Wernicke for kindly granting me permission to undertake this work and for his suggestions. Further, I thank the assistant, Dr. Lissauer, for his friendly and active support.

The aim of the work is to provide a macroscopic overview of the fibre connections of the occipital cortex as well as adjacent parts of the parietal and temporal lobes. Details and subtleties can be added to this work in the future.

Information on the white matter anatomy of the cerebral hemisphere is relatively scarce. In order to gain an overview of this field one has to go back to the beginning of the century, namely to Burdach's great work (1819; 1826; 1822), as fibre trajectories are only hinted at in more recent textbooks. The work by Meynert (1884) is difficult to understand and is not entirely evidence-based. Furthermore, the available case

reports are based on pathological specimens. Foundation work demonstrating the white matter anatomy in the healthy adult brain is entirely missing. However, in order to assign each case report its apt place in the system, the healthy human brain should be the reference for all other studies of pathological, foetal, and animal brains.

Identifying the directionality and trajectory of fibres within the white matter using only a single method is insufficient as each method has its inherent limitations. In order to overcome these limitations the results of at least two methods ought to be compared. For the current study three complementary methods were therefore employed:

1. The oldest method is “Zerfaserung” [post mortem blunt dissection], which was used exclusively by Burdach and honoured in particular by Meynert and his students. For the current work, I used brains that were treated with alcohol, yet were not too hardened. The method introduced by [Stilling \(1882\)](#) which uses “Holzessig” [wood vinegar] returns brilliant results for the brain stem but was, however, not suitable for the white matter of the hemispheres. The difference is that for this work it is not important to segment small parts of the brain into its fibre pathways but to relate the overall direction of fibres and connections between white matter bundles within a lobe. In contrast, blunt dissections return perfect results if the majority of fibres are running along the same direction, whilst the ubiquitous crossing fibres are not forming substantial bundles but are present in isolation or small numbers when piercing through the main pathways. In such cases they would fall apart smoothly or one does not notice them at all unless already familiar with them. Additionally, the presence of large fibre crossings can be identified using this method. However, it is not possible to follow with confidence the trajectory of the fibres beyond their point of convergence. Further, if fibres that thus far run in parallel start adhering to each other, as it is the case for callosal fibres towards the midline, this method will also fail. In both these cases tearing the tissue can create arbitrary artefacts. Coarse crossovers are not found in the occipital lobe and matting [occurs] only in the corpus callosum.

The most important drawback of the method is that it only gives us two-dimensional views. The direction and the width of a layer can only be identified with certainty if the layer is entirely destroyed. Therefore, blunt dissections are only for demonstration – in this case they are obviously invaluable to appreciate special organisation and relationships – but they are never sufficient as evidence in their own right.

2. The second method is the inspection of freshly prepared sections of specimens hardened in Müller solution and observed under direct light. These sections show the fibres or layers cut horizontally as pure white with only a hint of green. Areas where fibres are cut obliquely appear black-green and are darker in their shade than the dark green colour of the grey matter. Between these two extremes all shades of colours can be found depending on the cutting angle in relation to the direction of the fibres and whether

the majority of the exposed fibres were cut straight or oblique in regions of multiple fibre orientations. Additionally, differences in fibres, such as their width or the chemical nature of the myelin sheath, influence the tone of colour, so that the various layers can be clearly differentiated. If the brain is cut along different directions, the same layer can appear light or dark depending on the direction of the cutting.

3. The most important method, that allows magnification and inspection of individual fibres is the method introduced by Stilling and perfected by Meynert, where a series of sequential translucent and stained sections is prepared. To my knowledge only Pal's Haematoxylin is suitable as stain. In comparison, when sections of 1/20 mm thickness are prepared using Weigert's stain the white matter appears evenly blue, thus making it impossible to differentiate the various layers. The preparation of sections as thin as 1/10 mm for the whole hemisphere was only possible with great effort [as it requires], time and skill. Extremely rare is the successful transfer onto a glass plate after the specimen was exposed to the strains of staining and brightening. In contrast, 1/10 mm sections were suitable for staining according to Pal. Pal's stain has the advantage of staining some fibres very dark and deep blue, whilst other fibres are only lightly stained in brown-glow. Hence, it is possible to differentiate them. In the brainstem five different shades of colour can be distinguished. These shades depend partly on the variable thickness of the fibres and partly on the chemical properties of the myelin sheath. Another advantage of this method is, that sections stained with this method can be photographed. The photographs enclosed to this work [8 plates at the end of this publication] are an example of such sections and were provided by Dr. Lissauer.

The Picro-carmine stain allows identifying various white matter layers with the naked eye and the nuclei can be seen under the microscope. Structures that are usually coloured dark and blue by Pal's stain are stained yellow by picro-carmine. What appears light and brown using Pal is reddish with picro-carmine. The drawback is that in brain tissue, unlike peripheral nerves or cord, the axonal cones are not distinctly stained in red; therefore the individual fibres cannot be differentiated.

Note: When using Pal's stain for large specimens, such as a section of the whole hemisphere, a multitude of stratagems are required and negligence of each of them endangers the final result. I shall therefore carefully describe the method below.

The brain is removed from the skull as soon as possible after death, ideally in the winter and then preserved in Müller solution as a whole or only cut in halves (to avoid losing its shape). In the first few days, the solution needs daily changing. The specimen is ready to be cut after three to four months. Slices, cut as thin as the microtome allows, are dried by soaking them in diluted alcohol and pure alcohol, each for a period of 24 h. Slices are then immersed in celloidin solution and stuck to wooden plates. For the sections I used the largest Schanz microtome and an especially designed heavy knife. I did not cut under spiritus. Slices of 1/10 mm thickness can be

The cortex, whose white matter connections are to be described here, is delimited anteriorly by a frontal plane [fr], which passes tangential to the posterior end of the splenium

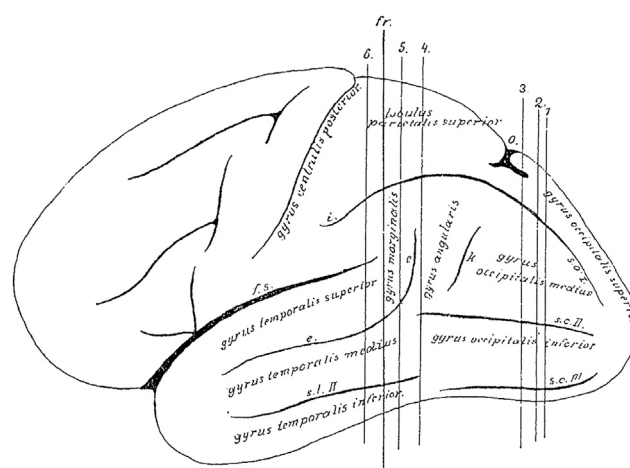


Fig. 1

On the convexity of the medial surface this plane cuts the most anterior part of the precuneus (Fig. 2). On the lateral convexity (Fig. 1) it cuts the gyrus at the end of the Sylvian fissure [supramarginal gyrus], whose most posterior cortical indentation extends into the depths. On the lateral convexity of this three-sided piece of brain, two sulci can be seen running dorso-ventrally [e,k], and three sulci running posterior-anteriorly [s.o. I–III], which all impact on the shape of the underlying white matter due to their depth. The more anterior sulcus [e] of the two vertical sulci is the ascending branch of the parallel sulcus [superior temporal sulcus] (Fig. 1e). This ascending branch lies entirely within the parietal lobe and is considered as part of the angular gyrus. The adjacent posterior vertical sulcus is the anterior occipital sulcus [posterior intermediate parietal sulcus] (k; see Wernicke (1881)). This sulcus considered representing the border between the parietal and the occipital lobes. This

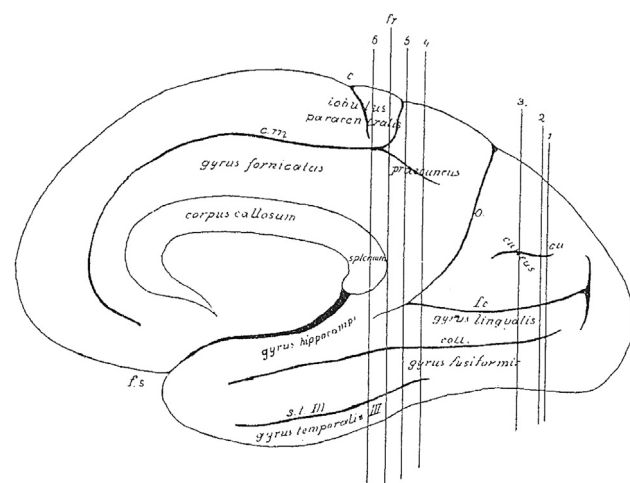


Fig. 2

sulcus can appear in different shapes. Usually, it continues ventrally into the continuation of the superior temporal sulcus [e] and thus gives rise to a second ascending branch of the latter. At times, however, it appears as a very short indentation without connection to any other gyri. It is, nonetheless, found in every brain and is readily identifiable, when following the occipito-parietal sulcus (o) on the convexity (Fig. 1) to the inferior transitional gyrus (above k) (Fig. 1) between the parietal and the occipital lobes. The opening of this gyrus is the anterior occipital sulcus.

Within the occipital lobe there are three deep sulci that are almost horizontal to each other before they separate anteriorly (Ecker, 1869). The superior/first occipital sulcus (s. o. I) is an extension of the intraparietal sulcus (i), which usually reaches the occipital pole, though interrupted. The middle/second occipital sulcus (s. o. II) reaches anteriorly towards the horizontal branch of the superior temporal sulcus (e). The inferior/third occipital sulcus (s. o. III) runs towards the second or third temporal sulcus. The inferior occipital sulcus often runs adjacent to the inferior convexity of the hemisphere and sometimes even at the basal surface. The middle occipital sulcus corresponds mostly to the lower occipital sulcus of Wernicke. Whereas both vertical sulci and the first horizontal sulcus are consistent and readily identifiable; the middle and inferior occipital sulci are often interrupted and branch off, and are therefore less clear.

The occipital lobe is delineated on the medial surface of the hemisphere (Fig. 2) by the occipito-parietal sulcus [o] separating the cuneus and precuneus, and by the calcarine fissure (f.c.), which adheres anteriorly with the above-mentioned sulcus [o]. Both sulci are rarely simple incisions. Usually, their stem forms a surface similar to the insula with secondary gyri. Nevertheless, this morphology is variable. The “posterior incision” of the occipito-parietal sulcus may extend many centimetres into the occipital lobe. Adjacent to the calcarine fissure a short gyrus extending rostrally-caudally can be seen superimposed on the top and bottom surfaces facing each other. In the depth of the fissure three vertical short gyri extend dorso-ventrally. Two of these can continue to the convexity of the sulcus and merge with the above-mentioned gyri; whereas the third sulcus, that is the middle or the posterior, never extends to the convexity. Such a short gyrus can reach at times the convexity and thus interrupt the fissure. The calcarine fissure, similar to the Sylvian fissure, has a superior (s) and inferior (i) opening (Fig. 3) but no posterior extension. Rather the base of the fissure becomes flattened and continues onto the medial surface of the hemisphere. This transition is to be seen in the forking of the fissure posteriorly. Within the cuneus, a gyrus parallel to the calcarine fissure extends rostrally-caudally [cu, Fig. 2].

In the precuneus, the horizontal, posteriorly directed extension of the sulcus callosus-marginalis (cm, Fig. 2) [cingulate sulcus] is important for white matter anatomy.

On the basal surface, the most important sulcus, which shapes the white matter is the collateral sulcus (coll., Fig. 3), which is the fifth sulcus to extend caudo-rostrally between the calcarine fissure and the inferior occipital sulcus and is variable in its extension in both directions. The medial occipito-temporal sulcus reaches very closely the occipital pole. In cases where the calcarine sulcus is a simple incision, the

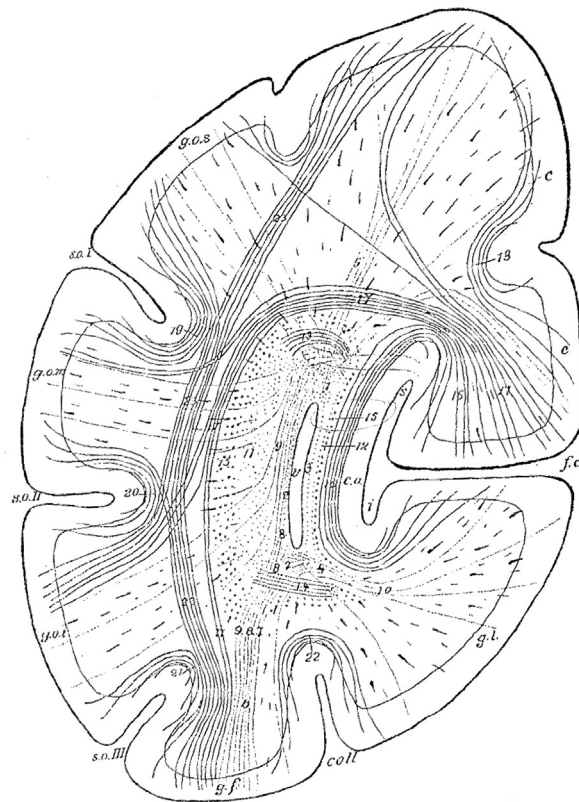
medial occipito-temporal sulcus can present a complex division.

The occipital horn begins to form as a canal with four walls, with thin dorsal and ventral walls and two-to-four-fold wider medial and lateral walls. Posteriorly, it rapidly loses its shape in all directions. Initially the loss is primarily in height more than width, so that it resembles almost a square before it loses its width and thus becomes a thin sulcus with its dorsal and ventral walls turned into edges. During its course it bends posteriorly in two directions. In its posterior part it bends gently along a vertical axis and thus its posterior end comes to lie closer to the medial plane than its aperture. In addition, it bends along a sagittal axis and becomes a slit, thus bringing the dorsal and ventral edges closer to the medial plane. From its posterior end a strip of ependyma, which retains its form, continues into the occipital white matter for a short distance. The double bend of the horn resembles the form of the hemispheric convexity and is due to the deep [occipital] notch close to the calcarine fissure. Apart from this, only the medial occipito-temporal sulcus has an impact on the shape of the occipital horn, by bulging its inferior surface a little in the middle part of the horn. All the other sulci, including the secondary deformations of the calcarine fissure, are of no importance to the shape of the occipital horn. These influence the width of the white matter only, and as is later to be seen, the thickness of the fourth and outermost layer, which lies immediately underneath the cortex and is referred to as the stratum proprium cortices. The deeper layers of the white matter are independent of the depth of these sulci.

The occipital horn lies closer to the basal surface than to the dorsal convexity of the hemisphere (Fig. 3); yet, it is equidistantly located between the medial and lateral surfaces. Nevertheless, due to the depth of the calcarine fissure it is separated from the cortex at the medial surface by a thin layer of white matter. The majority of white matter, on the other hand, develops at the lateral aspect of the occipital horn between the latter and the hemispheric convexity.

The fibres originating from the occipital cortex and coursing within the occipital white matter can be divided into two groups. Amongst these groups, one can again subdivide three groups: i) fibres that extend to subcortical centres and are considered as projection fibres or corona radiata (Stabkranz) (Meynert); ii) other fibres have their terminations in cortical areas and are therefore association fibres. Association fibres either interconnect intralobal cortical areas (short association fibres), or link the occipital cortex with the cortex of a different lobe (long association fibres); iii) the third group crosses the inter-hemispheric midline and might terminate in [contralateral] cortical or subcortical areas (callosal or commissural fibres).

This mass of the occipital lobe fibres is not a tethered bundle, but is rather organised into bundles and layers according to certain rules. These layers can be distinguished based on their direction, grouping and staining. The law of order is the following (Wernicke as cited above, p. 24): Every fibre reaches its destination via the shortest possible route, as far as this is in correspondence with embryological peculiarities of brain development. Thus, the following two conclusions can be reached: First, short fibres are located close to the cortex whilst longer fibres are located close to the ventricle.



Hinterhauptlappen. (Frontalschnitt.)

- | | | |
|-------------------------------|---|--------------------------------------|
| v. Hinterhorn des Ventrikels. | s. o. I. sulcus occipitalis superior. | g. l. gyrus lingualis. |
| f. c. fissura calcarina. | (fissura interparietalis.) | g. f. gyrus fusiformis. |
| s. Oberspalte. | s. o. II. sulcus occipitalis medius. | g. o. s. gyrus occipitalis superior. |
| i. Unterspalte. | s. o. III. sulcus occipitalis inferior. | g. o. m. gyrus occipitalis medius. |
| coll. sulcus collateralis. | c. a. calcar avis. | g. o. i. gyrus occipitalis inferior. |
| | | c. cuneus. |
-
- | | | |
|-------------------------------------|--------------------------------|-------------------------------------|
| 1.—10. forceps. | 17. stratum cunei transversum. | 21. stratum proprium s. o. III. |
| 11.—14. stratum sagittale internum. | 18. stratum proprium cunei. | 22. stratum proprium s. coll. |
| 15. stratum sagittale externum. | 19. stratum proprium s. o. I. | 23. stratum profundum convexitatis. |
| 16. stratum calcarinum. | 20. stratum proprium s. o. II. | |

Fig. 3

Second, fibres with roughly the same destination run in parallel or form bundles for a part of their common trajectory.

A second, generally valid biological law not to be ignored in the study of brain structure is the law of variability. There are no two brains that are identical in all their details. Variability is also observed in the arrangement and development of white matter anatomy. The cortex and the white matter are mutually dependent on each other. If a particular area of cortex is under-developed in a brain, then there is also a paucity of fibres originating from this area.

The occipital lobe fibres form four layers, which envelop the occipital horn like an onion skin from all sides except its opening. These layers, counted outwards from the medial to the lateral walls of the ventricles are (Fig. 3):

1. Layer of the corpus callosum: Forceps corporis callosi (1–10), a. pars magna superior (1), b. pars parva inferior (4)
2. Layer of the projection fibres: Stratum sagittale internum (11–14)
3. Layer of the long association fibres: Stratum sagittale externum (15)
4. Layer of the short association fibres: Stratum proprium cortices.
 - a. stratum calcarinum (16)
 - b. stratum transversum cunei (17)
 - c. stratum proprium cunei (18)
 - d. stratum verticale convexitatis.
 - α. stratum proprium fissurae interparietalis see also sulci occipitalis I. (19)
 - β. stratum proprium sulci occipitalis II. (20)
 - γ. stratum proprium sulci occipitalis III. (21)
 - δ. stratum profundum convexitatis (23)
 - e. stratum proprium sulci collateralis (22)
 - f. stratum proprium praecunei.

This layer is found in the region of the parietal lobe.

Another two bundles are closely located to the occipital lobe without joining its white matter system, namely:

5. Bogenbündel oder oberes Längsbündel, fasciculus arcuatus see also longitudinalis superior [superior longitudinal fasciculus]
6. Zwinge, cingulum.

Fibres originating from the occipital pole and surrounding areas bundle up in the middle of the white matter and run anterior-posteriorly. Far before the ventricular horn, these fibres group together into three concentric layers. The inner solid layer gives rise to the forceps. The remaining layers, which on axial sections look like a ring, form the internal and external sagittal layers. The forceps appears clearly as an independent layer, a few millimetres anterior to the other two layers. Thus, in the small stretch that includes the stratum proprium corticis, only three layers are evident. The three layers with a sagittal direction thicken anteriorly as new cortical fibres from all directions join them. At the caudal end of the posterior horn these fibres part like a funnel, so that all three layers equally cover the posterior horn.

5. Forceps corporis callosi

If there was no posterior horn, the occipital lobe was a solid structure, and the calcar avis did not reach deep into the white matter of the lobe, then the forceps would have the shape of a cone with its head at the occipital pole, where cortical fibres would gather like rays equally from all sides. However, now a bulge of the lateral ventricle, which forms the posterior horn, pushes into the forceps from the front, yet not along the axis of the cone, but rather closer to the lower surface. Therefore, the posterior horn is surrounded from all sides by longitudinally running callosal fibres and tears apart the lower part of the forceps. Due to the positioning of the corpus callosum above the ventricle, a major part of the forceps runs anteriorly over the posterior horn (Fig. 3.1). The medial and lateral surfaces of the occipital horn are covered by a thin veil of longitudinally directed callosal fibres (2, 3) with a stronger veil along the inferior surface (4).

The “large upper part of the forceps” flexes medially where the posterior horn opens up at the level of the quadrigeminal plate, in order to cross to the other hemisphere. As this part is at the height of the splenium from the very beginning, it is the natural confluence for all forceps fibres. All forceps fibres leave the cortex in a frontal plane, unless they already have entered the callosal layer, and therefore can be traced in their whole length to this point on coronal sections. Direct and unhindered access to the upper part of the forceps is only given to fibres from the cuneus and pre-cuneus, as well as fibres from the dorsal and lateral convexity of the hemisphere located above the intraparietal sulcus. These fibres not only join the forceps, but also dig deep into it, before they bend from a frontal plane into a sagittal direction. They thus divide the mass of sagittal fibres of the forceps into a number of tracts and layers. The layering is an expression of the interweaving of all callosal

fibres, which continues almost to the medial plane. Thus, callosal fibres from different parts of the occipital lobe lie next to each other. Until their insertion, fibres from the convexity of the hemisphere form a tightly packed, clearly differentiable fibre mass layer in the forceps (5).

On a frontal plane, the remaining callosal fibres originating from the cortex run inferior-superiorly along the occipital horn. Whether these reach their target at the lateral or medial surface of the occipital horn depends upon whether the cortical area they originate from lies lateral or medial on the sagittal plane through the middle of the occipital horn. This plane separates the lingual gyrus from the medial part of the fusiform gyrus at the basal surface. The fibre system originating from the fusiform gyrus – often a tightly packed layer, which is clearly differentiable from the rest of the fibres (6) – climbs vertically and breaks through both sagittal layers by dividing them into three parts. The inner-most part (7) runs at the basal surface of the posterior horn almost horizontal to it and bends slightly upwards, to insert in the yet-to-be-described small part of the forceps. A smaller middle part (8) bends in sagittal direction and strengthens the outer half of the forceps fibres that run sagittally on the inferior [part] of the posterior horn. The lateral largest part (9) runs along the outer surface of the posterior horn, adjacent and lateral to the thin layer of the horn. I shall call all callosal fibres at the outside of the occipital horn “outer forceps layer”. During its course along the outer surface of the posterior horn, this layer is continuously strengthened by fibres originating from the convexity underneath the intraparietal sulcus. These fibres run diagonally from the ventral convexity towards dorsal medial areas. Among them the most ventral fibres are close to a vertical direction. The more dorsal these fibres reach, the more horizontal they run, until they join fibres that cross to the upper part of the forceps directly above the intraparietal sulcus. They form small tracts, visible to the naked eye, that traverse both sagittal layers in the same direction as before and thus divide the latter in even smaller tracts. They then bend upwards in a vertical direction and join the ascending fibres. The whole layer thus becomes thicker as it ascends and bends from a vertical to a sagittal direction at the level of the upper part of the forceps. Also these fibres, like all callosal fibres, do not simply join from below or outside the already existing forceps system; they rather follow the same course of the callosal fibres [originating] from the dorsal cortex, i.e., they penetrate the forceps for a [certain] distance before bending in a sagittal direction.

The fibres of the sagittal veil which are directly adjacent to the lateral surface of the posterior horn (2) traverse diagonally along an anterior – superior [direction] and merge with the dorsal branch of the forceps. In the same way, the thickened bundle bends at the lateral aspect of the inferior occipital horn (8) more anterior and close to the opening of the occipital horn where it runs upwards and diagonally towards the front and then directly upwards to reach the same termination. The lateral sagittal veil shows greater variability. At times it can be clearly seen along the whole length of the occipital horn, in other cases it covers only the posterior part because its fibres bent upwards far more posteriorly and hence strengthen the layer of the vertical ascending fibre. The latter borders directly the ependyma.

The same position is not possible for those forceps fibres originating from the inner part of the fusiform gyrus, the lingual gyrus and the calcar avis at the medial surface of the occipital horn. This is due to the prominent calcar avis that bulges into the occipital horn and hinders a solid development of fibres that do not belong to the calcar avis. The entire forceps fibres originating from the lingual and fusiform gyri that should ascend vertically at this point are running longitudinally along the inferior medial edge of the occipital horn and thereby strengthen the medial half of the longitudinal fibres at the inferior occipital horn. Hence, this forms a cord-like tract, which thickens towards the front (4). Just before the anterior aspect of the calcar avis, directly behind the opening of the occipital horn, this tract has enough room to ascend as “small inner part of the forceps” from within the occipital horn. Once it reaches the roof of the ventricle, this tract bends inwards to join the larger upper part of the forceps and merge with the corpus callosum. The white matter of the fusiform gyrus is adjacent to the above-mentioned fibres that run inferior to the occipital horn (7), whilst the white matter of the lingual gyrus forms a denser layer (10) similar to the one from the dorsal convexity. The fibres from the thin sagittal veil at the inner surface of the occipital horn – the internal forceps layer (3), which are probably joined by callosal fibres originating from the calcar avis, merge anteriorly in the ascending part of the small forceps.

The entire inferior part of the forceps and the sagittal veil at the inner surface of the occipital horn show great variability. Both structures are mutually dependent: If the lower forceps is strongly developed, then the veil at the inner surface will be very fine to the point where it is difficult to appreciate it even at a high magnification and it might only consist of two or three fibre layers. In rare cases however, all of the inferior forceps vanishes and instead forms a tract merging with the veil, which develops as a relatively strong layer that uniformly covers the inner surface of the posterior horn. At times, the inner forceps does not ascend anterior to the calcar avis but ascends more posteriorly in a diagonal direction upwards and forwards.

All forceps fibres are characterised by a strong fibre diameter. The layers of the forceps stain rather dark with haematoxylin, and strongly yellow with picrocarmin.

6. Stratum sagittale internum

The stratum sagittale internum wraps around the forceps just as the forceps encases the occipital horn. The fibres of this layer differ from the fibres of the forceps for their smaller axonal diameter. This layer stains very light with haematoxylin, whereas picrocarmin-staining colours this layer in red compared to the surrounding layers. Fibres of this layer originate from the occipital lobe, seemingly from all areas of the occipital cortex, and continue anteriorly into the posterior part of the corona radiata. These fibres form the projection connections, namely the corona radiata of the occipital lobe. To reach their destination, they have to gather at the outer surface of the ventricle. Fibres originating from the occipital pole unify a few millimetres behind the beginning of the forceps as a solid tract that thickens as further fibres join

and runs anteriorly along a longitudinal direction. Once these fibres reach the tip of the forceps the tract funnels out and from here onwards encases the forceps from all sides in the shape of an anteriorly widening belt. On sections, fibres of the stratum sagittale internum were not traceable without interruptions along their entire trajectory from the cortex through the white matter. They can only be differentiated with clarity from other fibres, once they form a separate layer. Fibres at the inner surface of the forceps that run longitudinally towards the front (12) as well as fibres originating more anteriorly from the cuneus, precuneus, and lingual gyrus course towards the lateral surface of the forceps – still in the frontal plane – describing an arc around parts of the forceps that course dorsal and ventral to the occipital horn. Once these fibres reach the outside of the occipital horn they bend anteriorly in a longitudinal direction. On coronal sections, the upper parts of these fibres (13) cling to forceps fibres originating from the cuneus and the precuneus. Fibres from the lingual gyrus (14) run in parallel to the above described callosal fibres and course from the lateral to the medial surface in opposite direction from the base of the hemisphere towards the inferior part of the forceps (7).

As a consequence of this arrangement, the part of this layer that lies outside the occipital horn (11) becomes thicker, whereas the part on the inner side becomes finer as the calcar avis progressively penetrates the occipital horn anteriorly, such that it soon becomes only a microscopically visible veil. Eventually, the veil will tear apart just near the callosal bulge to allow the forceps to reach the median surface.

The most inferior fibres of the stratum sagittale internum run almost horizontal along their entire course towards the front. However, the more fibres originate dorso-anteriorly, the sharper their diagonal angle from a dorsal-posterior to an antero-inferior direction. In the parietal lobe the corona radiata runs eventually vertical on coronal section at the level of the tip of the pulvinar. Thus from here onwards they can be traced along their length on coronal sections.

As already mentioned, lateral to the occipital horn this layer is penetrated by fibres of the forceps that divide it in bundles of equal size. These bundles are visible to the naked eye. Close to the posterior arch of the caudate nucleus the middle part of this layer receives further additions from the yet-to-be-described stratum sagittale externum.

7. Stratum sagittale externum

The stratum sagittale externum (15) encloses the just mentioned layer in the same way the stratum sagittale internum covers the forceps. This layer consists mainly of fibres of large axonal diameter. Similar to the forceps, it stains very dark with haematoxylin, yellow with picrocarmin, and is thus clearly differentiated both from the stratum sagittale internum and the surrounding fibres. Whether the numerous fine fibres that cross the sections, which are visible at the level of this layer on coronal sections, are part of it or are just traversing it and strive towards the stratum sagittale internum, I have not been able to confirm with clarity. The latter seems more probable to me. Fibres of this layer originate from

the occipital cortex, seemingly from all its areas, and continue towards the temporal cortex except for a small portion. They form the long association tract between these cortices [inferior longitudinal fasciculus]. In order to reach their destination, which is the white matter of the temporal lobe, they all have to gather at the ventral aspect of the ventricle.

Posteriorly the layer appears as a thin belt, which envelops the stratum sagittale internum equally from all sides and initially describes the same course. These fibres could also not be traced continuously on their way from the cortex to their entrance into the stratum. It seems that these fibres, similar to those of the stratum sagittale internum, do not strive to their collection point like the fibres of the forceps which run vertically from the convexity of the brain on a frontal plane, in a manner similar to the branches of an apple tree to the stem. Rather, they radiate from posterior or diagonally from the cortex, anteriorly towards the ventricle like the branches of a pear tree to the stem. They therefore do not run in parallel to the forceps fibres towards the collecting layers but cross them like clasped fingers.

Fibres from the occipital pole and its neighbouring areas run anteriorly, longitudinal, and parallel to the ventral edge of the ventricle. The fibres underneath the occipital horn maintain their almost horizontal direction whereby they course towards the front and slightly descend in the temporal lobe. For the joining fibres it applies that the more the fibres originate from dorsal-anterior regions the more their direction changes from a dorsal-posterior to an anterior inferior descending direction. Hence, the most anterior fibres of this layer that originate from the convexity where the occipito-parietal sulcus cuts through, meaning from the first transitional gyrus, form an angle of approximately 30° with the most inferior fibres. In post mortem blunt dissections this layer therefore seems like a partially opened fan. At the inner surface of the occipital horn this layer, like the two layers previously described, thins out to a slender veil due to the deep penetration of the calcar avis. This veil behaves anteriorly similar to the thick covering on the lateral surface, in the sense that also here the fibres gradually take a vertical direction.

A consequence of this arrangement is that the stratum sagittale externum progressively tightens towards the base of the brain and forms a “track”, which becomes better defined towards the transition to the temporal lobe. The track consists of a solid foot with bilaterally attached side parts in a rounded right angle. This prominent inferior aspect of the stratum sagittale externum has been termed inferior longitudinal fasciculus by Burdach. Fibres from the cortex form a ridge-like attachment in the middle part of the occipital lobe at several points where callosal fibres penetrate the layer as thick tracts; this is the case dorsally towards the convexity, at the inferior edge of the stratum sagittale externum and towards the lingual gyrus. This attachment becomes very prominent and elongated in the lingual gyrus and has been named by Burdach as the internal basal bundle [inneres Grundbündel].

Once the stratum sagittale externum reaches the temporal lobe it quickly thins out by sending fibres to the cortex in all directions. When performing dissections, a large part of these fibres from the lateral aspect and the foot of this layer can be

followed into the first temporal gyrus. A smaller part reaches the second temporal gyrus, and the remaining fibres become insignificant and reach towards the temporal pole where they inseparably merge with the white matter of the temporal lobe and continue anteriorly. The most anterior fibres of this layer terminate in the pole of its lobe. In the anterior aspect of occipital lobe and the precuneus, sparse fibres descend diagonally from the medial surface of the occipital horn and after joining the cingulum they bend around the splenium and then continue with it – and in healthy brain they are inseparable from it-towards the temporal lobe.

Within the occipital lobe the stratum sagittale externum is divided into small, equal bundles by penetrating forceps fibres just like for the stratum sagittale internum.

A small amount of fibres of the stratum sagittale externum that lies lateral to the occipital horn does not reach the temporal lobe; instead the medial part of this subdivision segregates into numerous small bundles that are visible to the naked eye. These bundles are entangled like ropes and penetrate the stratum sagittale internum. They are differentiable within the latter due to the larger axonal diameter and their dark staining with haematoxylin. Both structures jointly reach the foot of the corona radiata.

8. Stratum proprium corticis

The majority of white matter between the stratum sagittale externum and the cortex has almost the same diameter in all directions as the three inner layers together. This white matter consists mainly of small association fibres, which originate and terminate within the occipital lobe. It is penetrated by long ranging fibres originating from the cortex and thence merging with the three inner layers. The short fibres mainly run in the frontal [coronal] plane, and thus interconnect dorsal and ventral or medial and lateral regions and only rarely do they interconnect directly adjacent cortical areas.

Three such fibrous tracts originate from the dorsal aspect of the cortical regions above the calcar avis.

The most important of these fibres is the *stratum calcarinum* (16), which consists of fibres that circumvent the calcar avis in its full extension, and the longest of which connect the cuneus to the lingual gyrus. In the white matter strips of the three above-mentioned vertical short gyri, which are placed on the insular ground of the calcarine fissure, this layer thickens into three strong bundles. Among these bundles the most anterior is rather prominent and partially reaches the base of the hemisphere. As a result of this filling of the “gyral comb”, the respective sulci do not appear at the bed of the calcar avis on the inner surface of the occipital horn. Anteriorly this layer reaches beyond the connection point of the calcarine fissure and the occipito-parietal sulcus into the temporal lobe and envelopes in a similar fashion the continuation of the calcarine fissure by connecting the cortex of the uncinate gyrus with the lingual gyrus.

The second layer originates from the dorsal cortical region of the calcar avis, the stratum cunei transversum (17). In contrast to the stratum calcarinum this layer only exists in the region of the cuneus and does not extend beyond the confluence of the calcarine fissure in the occipito-parietal sulcus.

The fibres of this layer originate together with those of the stratum calcarinum and initially run parallel with them over the dorsal calcar avis from medial to lateral. However, rather than bending downwards after the calcar avis they continue in the same direction above the dorsal part of the stratum sagittale externum and bend downwards on the other side of the latter to follow its lateral surface. On coronal sections cut through the posterior half of the occipital horn, this layer can be seen to reach the inferior border of the stratum sagittale externum: the more anterior the less these fibres reach inferiorly and the thinner the whole layer becomes until they eventually vanish in the region of the anterior occipital sulcus. Thus far it has not been possible to trace these fibres in isolation upon their exit from this layer along their trajectory through the stratum proprium convexitatis towards the cortex. They potentially reach the cortex of the whole convex region and part of the inferior occipital cortex, thus forming an association pathway between the cuneus and the convexity. It seems that these fibres have a straight direction only in their posterior and medial part along a medial to lateral course, however, more laterally and anteriorly, they bend out of the frontal plane and then continue diagonally along a posterior medial to anterior lateral direction. Thus the longest fibres of this layer might reach the dorsal parietal lobe and possibly the angular gyrus.

A subtler, yet at times quite prominent, analogous layer originates from the lingual gyrus and continues inferiorly around the stratum sagittale externum. In an ideal situation one can appreciate a fifth layer, which envelopes the latter stratum from all sides in the posterior frontal plane, where the occipital horn still is slit-like, between the stratum proprium cortices and the stratum sagittale externum.

Both layers, namely the stratum calcarinum and stratum cunei transversum, stain rather dark with haematoxylin yet less than the stratum sagittale externum. Therefore, they can be clearly differentiated from it and from the rest of the white matter.

The third layer, namely the stratum proprium cunei (18), which originates from the upper edge of the calcar avis, ascends perpendicular to the dorsal hemispheric margin and encapsulates the fissure of the cuneus that runs parallel to the calcarine fissure.

These three layers originating from the cuneus, seemingly form a joint system of short association fibres, which interconnect the cortex of the cuneus with the entire occipital cortex.

Similar to the region near the calcar avis, the space between the cortex and the stratum sagittale externum lateral to the occipital horn is filled by a stratum verticale convexitatis, which runs vertically in a dorso-ventral direction. Each of the three sagittal occipital sulci is enveloped by a system of gutter-like fibres [U-shaped fibres], which connect the gyri above and below the sulci; stratum proprium sulci occipitalis I s. interparietalis (19), str. Pr. S.o. II (20), str. Pr. S.o. III (21). A fourth system, the stratum proprium sulci collateralis (22), connects the lingual gyrus with the fusiform gyrus at the base of the brain. The more medial one reaches from the lateral aspect of the brain the longer the vertical fibres become. The fibres that follow the superficial strata propria of the sulci hurdle only one gyrus.

The deepest fibres directly abutting the stratum sagittale externum or stratum transversum cunei project along the whole height of the lobe and interconnect as stratum profundum convexitatis (23) the dorsal and ventral margins of the hemisphere.

This prominent vertical fibre system, the stratum proprium [verticale] convexitatis, is consistent across the whole posterior part of the cerebrum. Anteriorly it extends beyond the occipital lobe and gradually becomes thinner before its sharp boundary in the white matter strip between the post-central and the intraparietal sulci within the parietal lobe. Further inferior the most anterior vertical fibres encase the insertion point of the interparietal sulcus with the postcentral sulcus. They then reach the supramarginal gyrus from where they course anterior in the depth to join the association fibres of the insula that ascend from the operculum. In the temporal lobe, the most anterior fibres descend from the inferior aspect of the angular gyrus towards the second [middle] temporal gyrus and form the floor of the superior temporal sulcus, which at this point is often interrupted by a small vertical gyrus.

The stratum verticale convexitatis is also strongly developed in the monkey and has been described as fasciculus occipitalis perpendicularis by Wernicke (as previously cited, p. 23).

Similar to the sagittal sulci, both vertical sulci, namely the anterior occipital sulcus and the ascending branch of the superior temporal sulcus, are encapsulated by a very thin groove of longitudinally directed short association fibres.

In the precuneus, the layer of fibres adjacent to the cortex, namely the stratum proprium praecunei, also has a vertical direction and encapsulates the posterior elongation of sulcus callosi-marginalis in dorso-ventral direction. More medially located fibres bend anteriorly at their inferior terminations and join the dorsal part of the cingulum whose detailed description is yet outstanding. The deeper these fibres run, the farther anterior they penetrate the cortex of the gyrus fornicatus [the upper limb is the cingulate gyrus and the lower limb is the parahippocampal gyrus]. A third layer of vertically directed fibres is formed by the fibres previously described as belonging to the anterior medial part of the stratum sagittale externum and joining the descending part of the ventral cingulum reaching the temporal lobe. The second mentioned layer belongs to the anterior part of the precuneus, whereas the third belongs to its posterior part. Subsequently, fibres of the corona radiata follow that ascend towards the hemispheric margin.

In the anterior region of the occipital lobe and at the transition to the parietal lobe, where the stratum cunei transversum terminates, it remains a white matter system surrounded by the stratum proprium praecunei medially, the stratum verticale convexitatis laterally, and the stratum sagittale externum ventrally. This system abuts the superior part of the stratum sagittale externum like a roof ridge and consists mainly of fibres that run in a longitudinal cranio-caudal direction. This fibre system is only clearly visible on fresh coronal sections of a brain hardened in the Müller solution. It appears as a brighter area, which abuts the stratum sagittale externum like a cap and is distinguishable from the deep dark transverse cut of the latter, whilst it becomes gradually

indistinguishable towards the dorsal and lateral white matter of the stratum proprium corticis. It seems that this cap does not represent an association pathway in its own right but its curious appearance is rather owed to the fibres that pierce through here and then adhere to reach into the stratum sagittale externum and internum. It is possible that this area is also in relation to the most anteriorly bending fibres of the stratum cunei transversum. This is not noticeable in stained sections of a healthy brain.²

A similar smaller fibre system is present between the inferior part of the stratum sagittale externum and the stratum proprium sulci collateralis. A third system, at times in continuity with the just mentioned system, is found in the lingual gyrus close to the cortex of the calcar avis.

All these layers within the stratum proprium corticis, except the first mentioned stratum calcarinum and stratum cunei transversum, stain proportionally weak with haematoxylin.

With regards to the relation of size and form of all these white matter layers a look at the attached photographs will allow a better overview than any thorough description. Here, only the following will be mentioned, as it seems important with regards to pathology.

As mentioned above, the incision of the sulci into the white matter only affects the configuration of the outer most layer, the stratum proprium corticis, but only marginally the shape of the three inner layers (not even the stratum transversum cunei). Only the three layers of the calcar avis thin out to veil-like coverings. The medial occipito-temporal sulcus causes a concave invagination of the lower margin of the stratum sagittale externum; whilst the thickness of the stratum proprium corticis depends on the proximity of the cortical sulci to the stratum sagittale externum. At the medial surface of the brain this effect is visible in the thickening of the three above described gyri breves calcaris avis that form the stratum calcarinum. At the outer surface, the stratum proprium is pushed together by both vertical sulci of the occipital lobe, less so by the anterior occipital sulcus but [even] more by the ascending branch of the superior temporal sulcus. The stratum verticale convexitatis is especially thinned by the cortex of the most posterior protrusion of the Sylvian fissure. The thinner the outer layer, the easier a lesion that is originating from the cortex can reach the inner layers. A lesion progression from the cortex is thus easiest at the posterior end of the Sylvian fissure and underneath the second parallel sulcus, hence the region of the inferior parietal lobe.

Consequently, a superficial softening within this region can, depending on its depth, isolate the stratum sagittale externum or damage both the stratum sagittale externum and the stratum internum. This can cause transcortical syndromes such as optic aphasia (Freund) or apperceptive soul blindness [associative agnosia] (Lissauer) due to an interruption of the connections between visual and auditory centres. When the disconnection is present in association with a subcortical disturbance this causes hemianopsia. On the other hand, a lesion originating from the ependyma of the occipital horn may disrupts, apart from a small part of ascending

forceps fibres at the lateral surface of the occipital horn, the entire stratum sagittale internum in its function and thus cause pure subcortical hemianopsia without cortical or transcortical syndromes.

Additionally, two other tracts remain to be described, which are in close spatial relationship to the occipital lobe, especially to the stratum verticale convexitatis and stratum calcarinum. They do, however, not continue as part of the lobar white matter and are not in relation with its cortex. These are the arching fibres and the cingulum (Burdach). The arching fibres (respectively the fasciculus arcuatus or dorsal longitudinal fibres or longitudinalis superior) correspond to the stratum verticale convexitatis of the occipital brain in their course through the anterior parts of the brain. It is located in the depth of the dorsal gyrus of the Sylvian fissure, namely the operculum; its fibres extend dorsally approximately over half the height of the convexity. It consists of short association fibres, which connect neighbouring gyri with each other. In deeper layers, these association fibres bypass one gyrus at most. I doubt it contains long association fibres, which connect distant cortical areas. The deepest fibres of this tract running in the bed of the dorsal sulcus of the insula seem to have a special function. The direction of these fibres is always perpendicular to the direction of the corona radiata. In the region of the central gyrus and the dorsal part of the marginal gyrus these fibres run horizontally. At the transition point from the parietal lobe to the temporal lobe it bends downwards and joins the stratum verticale convexitatis whose anterior projections are identical with these fibres. Haematoxylin stains the arcuate fasciculus relatively light.

Along the medial aspect of the hemisphere the cingulum runs with a trajectory that is similar to the arcuate fasciculus. It originates underneath the callosal rostrum in the most posterior aspect of the inferior surface of the frontal lobe [subcallosal gyrus] as a thin wide layer that is inferiorly abutting to the corpus callosum. Initially, the fibres continue diagonally upwards and then form a bundle that bends dorsally around the genu and horizontally abutting to the corpus callosum directly underneath the cingulate gyrus. The cingulum runs along the entire length of the corpus callosum before it bends around the splenium and projects to the parahippocampal gyrus in the temporal lobe. When disregarding its frontal lobe trajectory, the cingulum can be segregated into a dorsal part, a descending part, and a ventral part. The cingulum consists of numerous small fibres that only stain lightly with haematoxylin and a compact tract of long dark staining fibres. The dorsal part of the cingulum includes the above-mentioned fibres that connect the cortex of the precuneus with the cingulate gyrus. The descending part separates the splenium from the anterior fibres of the stratum calcarinum from which it is distinguished by its dark haematoxylin staining. The inferior part is joined by anterior medial fibres of the stratum sagittale externum, which originate from the precuneus.

The above-mentioned tracts and layers can be visualised as detailed below by using the methods described above. The stratum verticale convexitatis can be easily demonstrated with blunt dissections where the cortex is broken off with a scalpel and the fibres are then removed in bundles using tweezers. This procedure permits to appreciate the sharp

² In paralytic brains this area appears as a bright patch in degenerative cortical changes. In these cases it is focally degenerated, never in its entire length (Lissauer).

anterior border of the layer and the anterior tracts of the temporal lobe that come together with the descending fibres of the arcuate fasciculus. If prepared carefully it is possible to visualise fibres that run through this layer and project medially along the white matter strip of the gyri. On fresh sections through a brain hardened in Müller solution, this layer appears light green on coronal or sagittal cuts and dark green on axial cuts. The stratum calcarinum and proprium sulci colateralis can be equally dissected, although, with more difficulty and only if they are well developed. They visualise similarly on fresh sections. I was not able to demonstrate the stratum transversum cunei with blunt dissections. On fresh sections of a well-hardened brain, however, this can be distinguished from stratum verticale convexitatis by its darker colour. The stratum proprium cunei is especially marked as a green-black transection on axial cuts of fresh sections. All these layers of the stratum proprium corticis are relatively strongly de-stained when using Pal staining. The strongest de-staining occurs for the stratum profundum convexitatis and a lesser effect is seen for the strata propria of the sulci; the stratum calcarinum and stratum cunei transversum remain dark blue, though still lighter than the stratum sagittale externum.

Blunt dissection is particularly good for the lateral surface of the stratum sagittale externum when the fibres of the cortex, which are perpendicular to them, are fully removed. The foot of the stratum sagittale externum is best demonstrated by dissecting from medial aspects where it is the basal bundle of Burdach and reaches close to the cortex of the lingual gyrus. When the calcar avis is reached the layer becomes too thin for further dissection. More anterior, however, the fibres from the precuneus that join the inferior part of the cingulum are well demonstrated. On fresh sections, this layer appears black on frontal sections and lighter on parietal sections especially in the dorsal parts due to the majority of descending fibres. It is distinguished from the stratum sagittale internum as well as its abutting cap by a different shade of stain. On axial cuts, however, the layer is light green and only on more dorsal cuts in anterior regions does it appear darker. The inner border between stratum sagittale externum and internum cannot be demonstrated with blunt dissection as the fibres of both layers have the same direction over long distances. The stratum sagittale externum is clearly distinguishable in all its parts from surrounding fibres when using Pal-stained sections – the stronger the de-staining of the section, the better the distinction. This stain is adequate for this layer. It stains strongly dark blue and can be followed under the microscope into its fine branches at the medial aspect of the occipital horn.

As mentioned above, the stratum sagittale internum cannot be clearly visualised by dissections beginning from the convexity, however, when starting from the medial surface its visualisation is possible when removing all callosal fibres. On fresh sections, this layer is distinguished from the stratum sagittale externum lateral to the occipital horn by a different shade of colour. Fibres that run transversely inferior and dorsal to the occipital horn are white on coronal cuts. When using Pal staining this layer stains only lightly and gains a brownish shade from which the dark blue callosal fibres, that traverse this layer, can be clearly differentiated. Picrocarmin stains this layer reddish compared to the surrounding

structures and shows its nuclei in a row along the penetrating callosal fibres.

The forceps is nicely shown in its entirety with blunt dissection; with the obvious exception of single fibres that penetrate the surrounding layers. On fresh sections the fibres that run underneath and lateral to the occipital horn towards occipital and dorsal regions penetrate the strata sagittalia. These fibres appear whitish on frontal cuts everything else appears black-green. On axial sections the association and commissural fibres are whitish and projection fibres are black-green. The Pal method stains these layers of the forceps almost as dark as the stratum sagittale externum.

It is easy to reveal the arcuate fasciculus with blunt dissection. On fresh coronal cuts, it appears as a dark slim ellipsoid – adjacent to the corona radiata – that sends a branch into the operculum; it completely disappears behind the Sylvian fissure. When using Pal staining, the arcuate [fasciculus] is not distinctly visible anywhere. The only change that becomes evident on coronal sections is that the region anterior to the caudal end of the Sylvian fissure where the arcuate is passing through is slightly lighter than the surrounding area after strong de-staining.

Blunt dissection nicely demonstrates the cingulum along its entire length including both its short and long fibres. On fresh coronal cuts, the long fibres appear as a black-green field that is abutting to the callosum and penetrates the cingulate gyrus. Behind the splenium it appears as a white-green thin cord with a dorso-ventral direction. On fresh axial cuts, it has exactly opposite colours. In the temporal lobe the cingulum disappears as an independent area. The Pal method stains its short fibres light, the long fibres dark blue, however, not as dark as the stratum sagittale externum.

Burdach (1822) has dissected the “inferior longitudinal fasciculus” with a branch towards the frontal pole. This is only possible, when coming from the stratum sagittale externum in the anterior temporal lobe to the posterior extension of the uncinate fasciculus, which covers the latter and connects the temporal pole to the orbital frontal lobe. Such a trajectory can be artificially produced with blunt dissection. The longest fibres of the uncinate fasciculus originate at the inferior lateral margin of the hemisphere, where the shortest fibres of the stratum sagittale externum, coming from posteriorly, terminate. This might therefore be seen as the area that best defines the border between the occipital and the temporal lobe. However, this could evoke the false impression of an uninterrupted trajectory of fibres through both bundles. On histological cuts it is immediately evident that this is just a deception, as both layers remain clearly distinct from each other. On coronal sections through the temporal lobe, the stratum sagittale externum becomes a slim horizontal dark line and disappears fully to the naked eye long before it reaches the temporal pole.

Meynert (as cited, page 41) believes that it is possible to follow the fibres of the anterior commissure into the occipital pole using blunt dissection. I was not able to replicate this. I could only follow fibre bundles of the anterior commissure up to the inferior margin of the cortex of the temporal lobe and I am convinced that the majority of these fibres end here (see also Wenicke as cited, page 86). A margin of error is given here, as fibres of the anterior commissure cross those of the stratum sagittale externum diagonally, thus permitting one to easily

get from one fibre layer into the other during dissection. Anterior commissure fibres can not be followed beyond the temporal lobe neither on fresh nor on histological coronal cuts.

Onufrowicz (1887) and Kaufmann (1887; 1888) have studied brains with congenital agenesis of the corpus callosum in which they found that the “tapetum” of the temporal and occipital lobes was present. Both authors could follow the tapetum anteriorly as a thick longitudinal fibre bundle, which they referred to as superior longitudinal fasciculus or arching bundle [Bogenbündel] of Burdach and believed it to be visible due to the absence of the corpus callosum. They thus inferred that the tapetum is not part of the corpus callosum, but rather the postero-inferior part of a large fronto-occipital fasciculus. This tract has thence been referred to as “fasciculus fronto-occipitalis” [superior fronto-occipital fasciculus] in textbooks by Obersteiner and Edinger.

I take the liberty to suggest here, that in order to avoid confusion already known structures of the brain should be referred to using the terminology introduced by Burdach until a full review of anatomical terms has been conducted. Burdach only refers to that part of the corpus callosum as tapetum, which runs towards the base of the brain, lateral to the lateral ventricle, and then continues anteriorly into the temporal lobe. Burdach refers to all remaining projections from the callosum to the occipital lobe as “forceps”. In more recent publications, even the fibres ascending at the lateral surface of the occipital horn and merging with the dorsal forceps are called tapetum. Both these layers correspond to each other and merge into each other at the opening of the occipital horn; yet, they can be differentiated from each other. The posterior fibres, which bend anteriorly and thus reach the temporal lobe, are the terminations of the tapetum. Fibres, that follow afterwards, of which the first descend straight [while] the later run towards the occipital lobe for a short distance in the dorsal forceps before descending, are part of forceps and constitute the anterior part of this layer that ascends towards the forceps along the lateral surface of the occipital horn. The border between both layers lies just behind the posterior arch of the caudate nucleus.

To my believe, both above-mentioned authors have mistaken the superior longitudinal fasciculus or arcuate fasciculus located close to the lateral convexity with the cingulum, which is located at the medial surface and separated from the arcuate by the corona radiata and the stratum sagittale externum. Owing to the absence of the callosum, the cingulum is positioned more inferior. The arcuate fasciculus³

³ Note from the authors: The original text here mentions *Bogenbündel*, which verbatim translates to *arching bundle*. When looking at Onufrowicz's original work, it becomes apparent that he equated the superior fronto-occipital fasciculus with Burdach's arcuate fasciculus: ‘This fibre tract may aptly be called ‘Fronto-occipitalis Associationsbündel’ [associative fronto-occipital fasciculus] or could be referred to as the true fasciculus longitudinalis superior. The ingenious Burdach [...] recognised, or rather guessed, this tract and called it the arcuate fasciculus or superior longitudinal fasciculus. Yet neither his [Burdach's] nor Meynert's descriptions of this tract are obvious and it is virtually impossible to prove it [fronto-occipital fasciculus] in the normal brain. We were able to appreciate this tract, located amongst callosal radiations in the normal brain only after comparison with our experiment of nature, in the case of agenesis of the corpus callosum.’ Translation in Forkel et al. (2014).

was not only hinted at by Burdach, as suggested by Onufrowicz, but was distinctly described by him. It is indeed easy to demonstrate this bundle in the healthy brain using blunt dissection or fresh cross-sections.

According to the description and the figures from both publications it can only be inferred that these fibres belong to the dorsal part of the cingulum and posteriorly merge with ascending fibres of the forceps. Though I have looked with utmost care, I was not able to follow any fibres from the dorsal part of the cingulum to the occipital lobe. The cingulate fibres are limited to the cingulate gyrus [Randgyrus des Balkens]. Unless they terminate within the anterior part of the precuneus or the descending part of cingulate gyrus, these fibres run in an arch around the splenium and reach the temporal lobe. Likewise, on fresh and stained sections it is impossible to demonstrate that cingulate fibres, which are clearly distinct everywhere, reach the occipital lobe.

Owing to Mr. Kaufmann's courtesy I was able to re-examine his anatomical preparations. I hereby arrived at the conclusion that this is not indeed an acallosal brain. The fibres of the corpus callosum are all present; they merely do not transverse to the contralateral hemisphere but rather remain in the same hemisphere and run anterior-posteriorly. Thereby producing a fronto-occipital bundle in the ‘acallosal brain’ that is completely absent in the healthy brain.

Such heterotopy of the corpus callosum is of no importance for the study of the normal brain anatomy, yet can be important for the study of its development.

More recently, Mingazzini (1890) indeed described a brain with complete callosal agenesis where the ascending forceps fibres and tapetum were also absent.

With regards to Hamilton's repetition of Foville's belief that the corpus is a cross-over of both internal capsules, the following is the case in the occipital lobe: callosal and projection fibres are clearly distinguishable from each other. Fibres from the posterior part of the foot of the corona radiata run ipsilateral towards the occipital lobe within the stratum sagittale internum and, to a smaller extent, within the stratum sagittale externum. [Also] there is no evidence that the forceps forms a commissure of both occipital lobes. For the time being, we cannot even speculate on the continuation of fibres after they come from the forceps on one side and transverse to the other hemisphere. They might reach totally different, anterior cortical regions or even reach the internal capsule. Both methods, namely blunt dissection and histology, fail to answer this question. In the future, this question might be addressed with unilateral lesion studies.

I believe that the widely accepted notion that the function of the corpus is to connect homotopical cortical regions (see Meynert as cited p. 41; Wernicke as cited p. 23) is wrong or at least incomplete. There is no evidence for this *a priori* opinion. Against this opinion stands the fact that callosal fibres entangle prior to reaching the midline. Most likely, fibres from certain areas of one hemisphere disperse in different directions after crossing the midline. There is no reason to assume that these fibres, instead of reaching their destination on the shortest possible way like all other fibres, reach the midline totally arbitrarily; and that they then so radically change their position that they come to lie smoothly in the same order next to each other as they did at the beginning.

The argument that Hamilton uses against previous scientists, especially Meynert, namely that it is impossible to follow a single fibre from one area of the cortex to the homologous area in the other hemisphere, also stands against Hamilton himself. It is equally not possible to follow a single fibre from the cortex to the internal capsule of the other hemisphere.

Generally, I agree with Schnopfhagen's (1891) interpretation of the corpus callosum as a "bed of association fibres, which connects structurally and functionally totally different regions of the hemispheres". It is beyond my judgment, if a minority of callosal fibres might reach the internal capsule in the frontal lobe as postulated by Hamilton. Schnopfhagen contested this opinion. In the posterior regions of the brain it seems that no callosal fibres enter the foot of the corona radiata.

Physiology postulates at least two tracts in the forceps. Assuming that the regions for focal vision are presented in the occipital lobes, a commissural tract within the forceps is to be expected, which connects the visual cortices. Additionally, there has to be a direct connection from the right occipital lobe to the left temporal lobe, which allows the naming of objects seen in the left visual field and which has to be disrupted in cases of optic aphasia of Freund. This tract is probably found within the forceps on the right side and within the tapetum on the left.

I ought to comment on a statement by Schnopfhagen on the straight occipital bundle of Wernicke. Schnopfhagen says (p. 102): "Wernicke describes a "straight occipital tract", a fibre bundle running from dorsal to inferior, which connects the second temporal gyrus (namely the *Pli courbe*, the dorsal part that is neighbouring onto the precuneus) with the fusiform gyrus [*Spindelwindung*]. A drawing of this tract, based on an axial cut through a monkey brain, is available in his book on brain pathologies (Fig. 19 ff). It seems to me beyond doubt that this "straight occipital bundle" is nothing but a plaited area at the convex lateral surface of the occipital horn."

It seems to me rather brave to reach an opinion "beyond doubt" based on schematic drawings of a third person, such as Wernicke's figure 19, from which a third party gained its assumptions. The "straight occipital bundle" is a collection of association fibres, which are evident in the monkey brain on horizontal cuts and especially on sagittal cuts where they appear as sagittally cut fibres. A triangular plaited region on axial sections, which is distinguishable from the rest of the fibre mass as a base of a gyrus at the convex lateral surface of the wall of the occipital horn exists neither in the monkey nor in adult human brain. In the human brain, the association fibres of the stratum profundum convexitatis are so prominent that individual fibres from the callosum, the corona radiata or long association fibres running towards inner layers fully disappear within this system.

The following conclusion do actually not belong here but are rather destined for the end of the work dedicated to the entire white matter anatomy of the cerebrum. Meynert's theory about the development of psychiatric activity is based upon the anatomical assumption that each part of the cortex is in direct anatomical connection to each other, such that between any two random cortical regions association tracts can be carved out (Meynert, p. 138). My research thus far does not support such an assumption as a general rule. The

occipital lobe has only one long association tract, namely the stratum sagittale externum that connects to the temporal lobe [inferior longitudinal fasciculus]. Possibly, there might also be some minor connection via the anterior fibres of stratum transversum cunei between the cuneus and the posterior part of the parietal lobe. Apart from these there is no evident connection with the parietal or frontal lobes (neither their convexity nor their medial surface), which would be comparable to the dimensions of the connection reaching the temporal lobe. Likewise, apart from the temporal lobe, there is no significant long association tract between two physiologically distant brain regions. Provable connections are limited to the vicinity and even the longest of these stay within the borders of each lobe. Any long [interlobar] fibres would therefore have to be relatively few and isolated.

However, this is rather different for the temporal lobe. The temporal lobe has a strong connection with the occipital lobe via the stratum sagittale externum. An important, though less prominent connection to the frontal lobe is via the uncinate fasciculus. The cingulum connects the temporal lobe to the precuneus, paracentral lobe and the part of cingulate gyrus that lies above the callosum. The cingulate fibres might even reach the frontal lobe. The temporal lobe is in connection with the parietal lobe via the posterior part of the arcuate fasciculus or the anterior fibres of the stratum verticale convexitatis. Additionally, it is the only lobe to have commissural fibres, meaning that for the anterior commissure is true what is not the case for the callosum: fibres in both hemispheres run in the same way without crossing or entangling.

In comparison to these very prominent associations, the corona radiata of the temporal lobe is relatively insignificant. Apart from the fornix, which connects to the mammillary bodies and possibly also to a cortical area, only a small amount of fibres enters the internal capsule.

This arrangement is possibly the anatomical expression of the psychological fact that language is of utmost importance for human thought process. Words and sounds have direct anatomical connections with all primary cortical areas for sensory perception, whereas those areas themselves are only indirectly connected via the speech centre. All separate parts of thought, which eventually are composed from the memory of various sensual perceptions, are in essence connected by the medium word, which expresses the thought.

Thus the anatomical study of the brain makes one understand the incredible power the word has for human beings, in their every day life, but also in hallucinations of the mentally ill, and the confabulations of the hypnotised. This physiological arrangement of the thinking organ might be the reason for the phenomenon that a congenital blind person is able to develop all higher cognitive functions despite the lack of the most noble of senses, whilst deaf-mute people only on rare occasions can rise above the level of an animal.

9. Annotations to the enclosed photographs [1–8]

The enclosed photographs are taken from specimens stained with the Pal method and are represented according to their

natural size. Vertical lines 1–6 in Figs. 1 and 2 represent the approximate level of each cut.

1. The cut is located approximately 25 mm anterior to the occipital pole. Apart from atypical sulci, this cut shows the transection of the beginning of the three occipital sulci (as above I, II, III) on the lateral convexity and the collateral sulcus on the inferior surface (coll.). The medial aspect shows the calcarine fissure (f.c.) with its prominent dorsal and inferior gyri. These are evident even with strong destaining of the specimen due to the white matter strips running within the cortex as well as the sagittal running sulcus of the cuneus (cu.). The posterior horn is not yet visible on this section. Also the callosal fibres have not yet united to form a distinct layer. The medial aspect of the white matter offers only two layers that are concentric and form a triangle with the dorsal tip and a ventral base. This triangle is roughly equal to the size of the calcar avis. The light layer in the middle is the stratum sagittale internum (1), the lateral darker layer the stratum sagittale externum (2). The white matter towards the cortex, which is the white matter of the occipital lobe, the stratum profundum convexitas (10) is stained relatively light, the strata propria of the cuneus (5) of the three occipital sulci (6,7,8) and the sulcus collateralis are stained slightly darker. Even darker, yet lighter than the stratum sagittale externum, is the stratum calcarinum (4), which is located between the latter and the cortex of the calcar avis. The same shade is evident for the stratum transversum cunei (3), which jointly originates with the latter fibres from the cuneus and runs dorsal and lateral to the stratum sagittale externum. This [3] can be followed as a slim grey strip on the lateral aspect of the stratum sagittale externum to the inferior lateral margin. Hence, this plane shows a total of five encapsulated layers, which differ in their staining intensity and can therefore be separated.
2. This cut is located approximately 1 mm anterior to the previous one. This section shows the forceps as a dark stipe that is thin dorsally and widens inferiorly. It is located in the middle of the homogenous stratum sagittale internum as described on the previous section.
3. This cut is located approximately 5–10 mm anterior to the previous one and approximately 30 mm away from the occipital pole. On the medial margin this section demonstrates the calcarine fissure (f.c.) underneath a deep sulcus of the cuneus. Compared to the previous section, the foundation of this fissure is deformed from a wide base to a thin ditch. On the dorsal cortex of the calcar avis, as well as on the ventral cortex, a secondary sulcal development is evident. The inferior margin of this section shows the section through the well-developed collateral sulcus (coll.). On the lateral convexity there are five evident transverse sulci. The four inferior ones of these equal the three longitudinal occipital sulci with the dorsal one forking into two branches (s.o. I–III). The calcar avis is underdeveloped which is compensated for by a dominant protrusion of the occipito-parietal sulcus into the white matter of the occipital lobe. This sulcus merges with the main sulcus (o.), which infringes from the convexity and then runs posterior. On this section the posterior horn can already be

appreciated as a triangle with a small base, the medial and inferior borders concave and the lateral border convex (v.). The indentations of the medial and inferior borders are necessitated by the intrusion of the calcarine fissure and the collateral sulcus. Amongst the medial white matter layers the forceps (1) has a prominent dorsal part and a less prominent spoon-tongue-shaped inferior part that is reaching into the lingual gyrus. Medially to the occipital horn the single layers cannot be differentiated due to the overall dark staining on the section. The bright area at the inferior half of the forceps fibres located lateral to the occipital horn is not a genuine appearance but originates from an error during the cutting of this section. The reason for this is the presence of a vessel in the ependymal of the occipital horn, which damaged some fibres along several sections. The stratum sagittale internum (2) can be appreciated as a bright area dorsal, lateral and inferior to the forceps. It then continues as a beak-shaped protrusion into the lingual gyrus. Medial to the occipital horn the layer has become too thin for examination with the naked eye. The same applies to the dark stained stratum sagittale externum (3). The inferior aspect of it [3] is thinned and bend due to the collateral sulcus, whilst the intruding sulci only cause a slight curvature on the lateral aspect. It already becomes evident on this section that the infero-lateral aspect of this layer is more dominant than other parts.

The stratum profundum of the convexity (10) is lightly stained whilst the layers of the sulci (6, 7, 8, 9) are easily differentiated due to their darker stain as we have already seen on previous sections. Exceptionally dark – nearly comparable to the stratum sagittale externum – are the stratum calcarinum (5) and the stratum transversum cunei (4) whose common origin in the cuneus is clearly visible. At the lateral dorsal border of the cortex, the stratum transversum cunei forms a helm-shaped cap, which is formed by its dorsally projecting fibres. It thence continues as a slim stripe at the lateral surface of the latter with the result that it nearly reaches its latero-inferior border.

4. This cut is located approximately 30 mm anterior to the previous one and approximately 60 mm away from the occipital pole. The majority of this section is not located in the occipital lobe anymore. The lateral aspect shows the dorsal parietal lobe (I) and underneath it the inferior part of the angular gyrus (II). The medial aspect shows the precuneus (VIII) and – as the most anterior part of the occipital lobe – the anterior termination of the cuneus, which is thinning to remain as slim stripe (VI). The main fissures of the surface anatomy that can be appreciated here are the intraparietal sulcus (i) and at the inferior margin of the hemisphere the third occipital sulcus. The latter might already be referred to as the third temporal sulcus here (s.o.III). The collateral fissure (coll.) is again visible adjacent to the inferior part of the stratum sagittale externum. On the medial aspect the fissure calcarina (f.c.) and the occipito-parietalis sulcus (o.) are abutting just after they have merged. The cross-section of the precuneus shows the posterior elongation of the callosomarginal sulcus (cm).

The occipital horn in this particular specimen is rather wide in its anterior half. In comparison to the previous section, it gained in width and formed a prominent dorsal surface, which is protruding convex into the ventricle dorsally due to the protrusion of the dorsal part of the forceps. The dorsal part of the forceps (1) gained significantly in size and continues at the lateral surface of the occipital horn (2) into the vertically ascending fibres. These fibres appear as longitudinally cut under the microscope (compare figure 3 and 9). The forceps fibres underneath the occipital horn are cut longitudinally where they reach for the stratum sagittale internum and are cut transverse where they are close to the ventricle (Fig 3.7). The inferior part of the forceps (4) is still located at the inferior margin of the occipital horn. The connection between this and the dorsal part is formed by a thin layer of fibres that are cut transversely and that run along the inner surface of the occipital horn, namely the medial forceps layer (3). The stratum sagittale internum (5) disappeared where the calvar avis is penetrating the white matter and is not visible in this specimen under the microscope. The part of it that is located lateral to the occipital horn is formed by transversely cut fibres, whilst its fibres dorsal and inferior to the forceps are cut longitudinally and constitute the addition to this layer that comes from the cortex of the medial occipital lobe. The beak-like extension of the stratum into the gyrus lingualis, which was already present on the previous section, can still be visualised here. The beak appears as a transversally cut fibre bundle under the microscope. The stratum sagittale externum (6) is similar to the internum in its shape. Its inferior part is further thinned and bend due to the collateral sulcus. On the lateral aspect it is already visible to the naked eye that the layer is disappearing due to the various penetrations of thin bundles of fibres designated to reach the forceps. In the inferior part the fibres are transversely cut whilst in the dorsal part they are cut aslant. When comparing this section to the previous one, the formation of bundles from forceps fibres is evident in the region between the stratum sagittale internum and the externum. The strata propria of the interparietal sulcus (10) and the collateral sulcus (12) are clearly distinct from deep layers of the cortex (9), as the latter is stained lighter. The dorsal part of the stratum sagittale externum is covered by a cap that appears darker compared to the surrounding fibres. These lighter fibres are the anterior remnant of the stratum transversum cunei, which will disappear more interiorly together with the cuneus.

5. (Enlargement $\frac{9}{8}$) This cut is located approximately 5 mm anterior to the previous, approximately 65 mm away from the occipital pole, and only few millimetre before the posterior part of the corpus callosum. This section therefore covers entirely the parietal lobe. The remnant of the cuneus that was still visible on the previous section has now disappeared and made room for the descending part of the cingulate gyrus (VIII). Dorsal to this the precuneus (IX) is cut along its largest diameter. With regards to the fissures on the convexity, the interparietal sulcus (i) is cut diagonally and the ascending branch of the parallel sulcus (e.) is cut longitudinally. Underneath the latter one can appreciate the transversely cut second and third temporal

sulcus. On the basal aspect one can see the collateral sulcus again the indents the stratum externum and on the border to the inferior medial aspect the anterior and shared part of the calcarine fissure with the occipito-parietal sulcus (f.c.).

The basal aspect is reduced in size in relation to the other two as well as in its absolute diameter and its direction got closer to the medial surface, meaning it is more vertical. The convexity on the other hand is approaching the hemispheric midline inferior just as it always has done superiorly. As a consequence of these dramatic changes in the arrangement of the gross anatomy the subcortical anatomy of the white matter and the occipital horn is rendered. The occipital horn gained in width and height and has four walls as it did on the previous section. Amongst these walls the inferior one is very thin and corresponds to the lateral part of the inferior wall from the previous section. The medial part with the adjacent collateral sulcus is now the medial wall. The dorsal wall is, similar to the previous section but more prominently indented due to the dorsal forceps part. This section shows the transition of the occipital horn into the lateral ventricles.

The dorsal (1) and ventral (2) forceps part gained in volume. The ventral part projects dorsal along the inner surface of the occipital horn and is therefore only separated from the dorsal part by a thin gap. The fibres of the inner forceps layer merged with it. Additionally fibres originating from the inferior convexity are joining the forceps via the medial wall of the occipital horn. Likewise the dorsal forceps part gains volume from the now prominent layer of fibres that are ascending vertically along the lateral surface of the occipital horn (3). Both additions are cut longitudinally on this section whilst all other forceps fibres, including the small bundle at the inferior border of the occipital horn, are cut transversely.

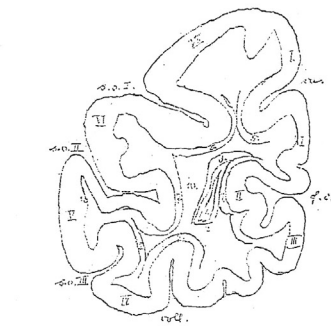
Both sagittal layers migrated away from the medial surface of the dorsal forceps to allow the fibres destined for the splenium to pierce through. The majority of the stratum sagittale internum (4) is located lateral and to a smaller extent inferior to the occipital horn. On this section one can appreciate medial cortical fibres running dorsal and ventral to the forceps towards this layer (5). The stratum sagittale externum (6) tightened towards its base ventral to the occipital horn. Medial and dorsal to the dorsal forceps part no fibres of this layer are seen on this section. The directionality of the fibres is exactly the same as on the previous section. With the calvar avis the beak-like protrusions of both sagittal layers vanished. The white matter of the cingulate gyrus, namely the cingulum, is cut longitudinally (7) at its medial aspect where it descends behind the callosum. The cingulum is stained dark here and therefore easily differentiated. The cortical white matter layers are prominent and slightly darker in there staining. These include the strata propria of the sulcus collateralis (10), the precuneus (8), and the fissure interparietalis (9).

It should be noted that the dorsal and lateral areas of this specimens are generally darker stained compared to the rest. The reason for this irregularity might be found in the irregular hardening of the brain as well as the very strong (and therefore not necessarily even) de-staining necessitated by the intent to photograph the sections.

6. This cut is located approximately 10 mm anterior to the previous, approximately 75 mm away from the occipital pole, and anterior to the brain structures of this examination. The intent is to indicate the subsequent white matter trajectory. This section shows (i) the posterior part of the central sulcus (I) dorsally, (ii) the remnant of the Sylvian

fissure (f.s.) laterally, and (iii) the callosum, fornix and the posterior part of the hippocampus medially. With regards to the sulcal anatomy, apart from the Sylvian fissure, the interparietal (i) and parallel sulcus (e.) as well as the second and third parietal sulci (s.t. II and III) are seen on the lateral convexity. On the medial surface one can appreciate the

Phot. 1.



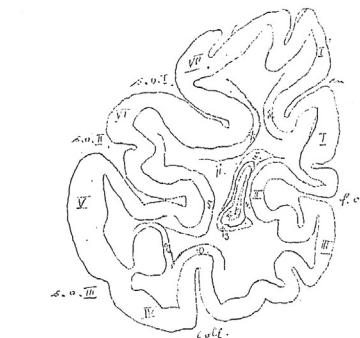
s. o. I. sulcus occipitalis superior.
s. o. II. " " medius.
s. o. III. " " inferior.
coll. sulcus collateralis.
f. c. fissura calcarina.
cu. sulcus cunei.

I. cuneus.
II. calcar avis.
III. gyrus lingualis.
IV. " fusiformis.
V. " occipitalis inferior.
VI. " " medius.
VII. " " superior.



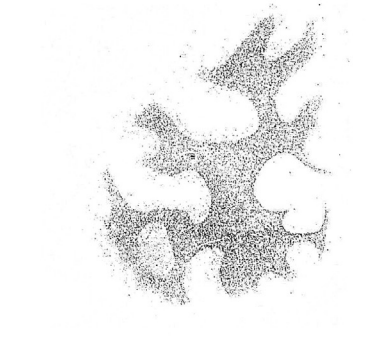
1. stratum sagittale internum.
2. " " externum.
3. " transversum cunei.
4. " calcarinum.
5. " proprium cunei.
6. " sulci occipitalis I.
7. " " " II.
8. " " " III.
9. " " collateralis.
10. " profundum convexitatis.

Phot. 2.



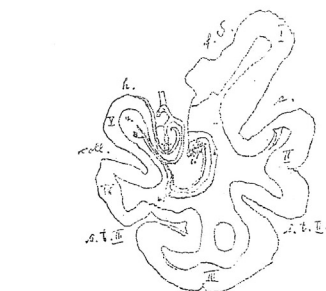
s. o. I. sulcus occipitalis superior.
s. o. II. " " medius.
s. o. III. " " inferior.
coll. sulcus collateralis.
f. c. fissura calcarina.
cu. sulcus cunei.

I. cuneus.
II. calcar avis.
III. gyrus lingualis.
IV. " fusiformis.
V. " occipitalis inferior.
VI. " " medius.
VII. " " superior.



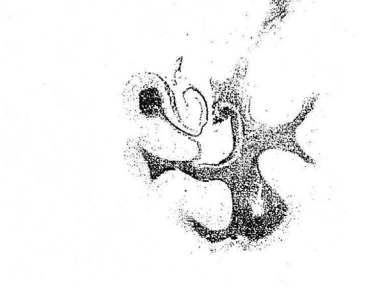
1. forceps.
2. stratum sagittale internum.
3. " " externum.
4. " calcarinum.
5. " transversum cunei.
6. " proprium cunei.
7. " sulci occipitalis I.
8. " " " II.
9. " " " III.
10. " " collateralis.
11. " profundum convexitatis.

Phot. 8.



v. cornu inferius ventriculi.
f. S. fissura Sylvii.
e. sulcus parallelus.
s. t. II. sulcus temporalis medius.
s. t. III. " " inferior.
coll. sulcus collateralis.
h. " hippocampi.

I. gyrus temporalis superior.
II. " " medius.
III. " " inferior.
IV. " fusiformis.
V. " hippocampi.
VI. cornu Ammonis.



1. tapetum.
2. corona radiata lobi temporalis.
3. stratum sagittale externum.
4. cingulum.

The occipital horn transitioned into the descending part of the cella lateralis of the lateral ventricle, which is only separated from the cortical surface ventrally through the fimbriae of the fornix (12) that are running into the hippocampus. The fibres of the forceps are freed from the white matter and the cortex, which were still separating it from the midline on the previous section, and are now located dorso-medially to the

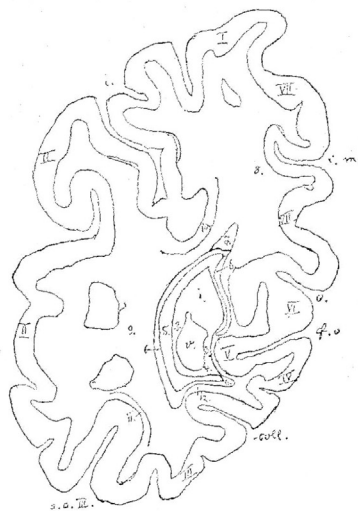
ventricle in the splenium. The dorsal (1) and ventral (2) part of the forceps can still be separated. The vertically ascending fibres (3) lateral to the ventricle are not part of the forceps in this section anymore but belong to the tapetum of the temporal lobe. The tapetum has a posterior protrusion and is thinned due to the descending part of the caudate nucleus, which is not visible on this section. The dorsal region of the tapetum is filled with cortical fibres that pierce the next layer (**). The fibres of the stratum sagittale internum (4) are all collected on the lateral surface of the ventricle and lateral to the tapetum. The dotted appearance in the middle of this layer



(4*) is due to merging with other bundles from the lateral aspect of the stratum sagittale externum that are still darker and therefore differentiate from the fibres of the stratum sagittale internum. Under the microscope each of these bundles shows a rope-like twist around its own axis. The whole layer represents the posterior part of the base of the corona radiata and gains fibres ventrally from the temporal lobe and dorsally from the parietal lobe. The stratum sagittale externum (5) is now limited to the ventral part of the ventricle in

the region of the temporal lobe and thins out as it sends fibres off to the temporal cortex. Towards the hippocampal gyrus, the stratum sends a protrusion that is long, thin, and a still indented by the collateral sulcus. The termination of this protrusion is joined by the cingulum. Lateral to the ventricle it extends barely until the Sylvian fissure as its demarcation fades away. The elongations of the corresponding layers of the stratum vertical convexitatis are the strata propria of the interparietal (9) and parallel sulcus (11) as well as the white

Phot. 4.



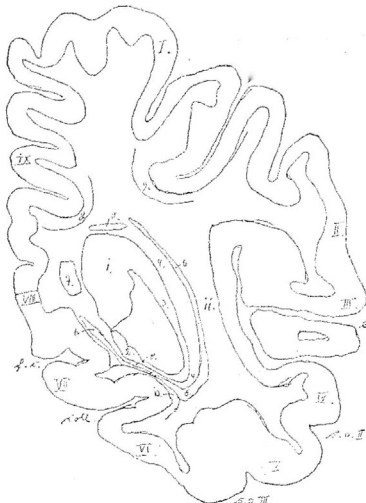
v. cornu posterior ventriculi.
i. fissura interparietalis.
s. o. III. sulcus occipitalis inferior.
c. u. Verlängerung des sulcus callosi-marginalis nach hinten.
o. fissura occipito-parietalis.
f. c. „ calcarina.
coll. sulcus collateralis.

I. lobus parietalis superior.
II. gyrus angularis.
III. „ foveiformis.
IV. „ lingualis.
V. calcar avis.
VI. cuneus.
VII. praecuneus.



1. pars magna superior foricis.
2. Aeusere aufsteigende Forcepsfasern.
3. Innere quergeschnittene „
4. pars parva inferior foricis.
5. stratum sagittale internum.
6. „ „ externum.
7. „ „ transversum cunei.
8. „ „ proprium praecunei.
9. „ „ verticale convexitatis.
10. „ „ proprium fissurae interparietalis.
11. „ „ sulci occipitalis III.
12. „ „ „ collateralis.

Phot. 5.



v. cornu posterior ventriculi.
i. fissura interparietalis.
e. sulcus parietalis.
s. o. II. sulcus temporalis medius.
s. o. III. „ „ inferior.
coll. sulcus collateralis.
f. c. fissura calcarina.

I. lobus parietalis superior.
II. gyrus marginalis.
III. „ temporalis superior.
IV. „ „ medius.
V. „ „ inferior.
VI. „ fusiformis.
VII. „ lingualis.
VIII. „ fornicatus.
IX. praecuneus.



1. pars magna superior foricis.
2. pars parva inferior foricis.
3. Aufsteigende Forcepsfasern.
4. stratum sagittale internum.
5. Zuzüge zu 4.
6. stratum sagittale externum.
7. pars descendens cinguli.
8. stratum proprium praecunei.
9. „ „ fissura interparietalis.
10. „ „ sulci collateralis.
11. „ „ verticale convexitatis (profundum).

matter of the Sylvian fissure (10), which are all darker stained. The cortex is closely approaching the corona radiata of the occipital lobe by a few millimetres at the deepest area in the Sylvian fissure. Dorsal to the splenium a transverse cut of longitudinal fibres shows the cingulum (7) reaching into the cingulate gyrus. On the previous section the cingulum was cut along its descending length. The lighter area between the layers of the interparietal sulcus and the Sylvian fissure indicate the location of the superior longitudinal bundle or arching bundle (6). Similar to the previous section, the dorsal and lateral areas of this specimen are darker stained compared to the rest.

7. This section is taken from a different series from an atrophic female brain of an elderly lady. This section clearly demonstrates the triple layering of the occipital horn, including its internal surfaces, and the area between the horn and the calcar avis (VI.). This section is also a coronal cut and is to be placed between the previous sections 4 and 5, only slightly anterior to the section 4. The corresponding photography demonstrates the medial aspects in a roughly fourfold enlargement and corresponds to the square that is indicated in the schematic diagram of the same section. The stem of the cuneus (VII.), which was still visible on the surface on section 4, withdrew here into the depth of the occipito-parietalis sulcus (o.) and the calcarine fissure (f.c.). The cross-section of the white matter is identical to section 4, only that it is possible to identify the medial forceps layer (2), the stratum sagittale internum (5), and externum (6) along the entire cross-section of the medial aspect of the occipital horn and between the horn and the calvar avis. These can all be seen with the naked eye.

The cross-section of the occipital horn is squared with the dorsal surface being formed by the dorsal forceps, the medial surface by the calcarine fissure, and the inferior surface by the collateral sulcus. The dorsal forceps (1) is rather prominent,

the ventral forceps rather weak (4), whilst the medial forceps layer (2) is relatively strong and equally thick as the lateral layer (3). The majority of fibres of the stratum sagittale internum (5) are collected lateral to the ventricle, whilst the fibres of the externum are collected ventral to it (6). However, fibres of the latter are still to be found in the lingual gyrus and to a smaller degree in the stem of the cuneus. It is possible to trace a veil from both layers across the medial surface of the occipital horn with the naked eye.

8. This photography shows a coronal section through the temporal lobe of a brain that suffered a stroke. As a consequence of the stroke the occipital cortex and a part of the temporal cortex, especially the first temporal gyrus, ipsilateral to the lesion are damaged. The level of this section is comparable to section 5. The brain stem was removed prior to hardening this specimen. A ramification of the removal is that the temporal lobe anatomy was altered and the cortex shifted more medial. The area of the cut showing the corona radiata of the temporal lobe is bend medially and almost reaches the hippocampus, which caused the unusual form of the lateral horn.

The convexity of this section shows the Sylvian fissure laterally and the sulcus hippocampi (h.) medially. Within the section the following structures are evident: the parallel sulcus (e.), the second and third temporal sulci (s.t. II. and III.), and the collateral sulcus (coll.), which indents the lateral horn from the inside towards the eminentia collateralis Meckelii. The majority of the first temporal gyrus and some of the second temporal gyrus are affected by the stroke. From the second temporal gyrus a bright layer of degenerated fibres runs towards the white mater ditch.

From the occipital horn a small remnant of the tapetum is present (1) lateral to it lies the well-maintained corona radiata of the temporal lobe (2) whose propagation into the elongation stratum sagittale internum is cut off. The very prominent dark

Phot. 7.



v. cornu posterius ventriculi,
i. fissura interparietalis,
o. sulcus occipito-parietalis,
f.c. fissura calcarina,
coll. sulcus collateralis,
s.t. III. sulcus temporalis inferior.

I. Erste Übergangswindung.
II. Zwerch.
III. gyrus temporalis inferior.
IV. „ fusiformis.
V. „ lingualis.
VI. calcar avis.
VII. Stiel des cuneus.
VIII. praecuneus.



1. pars superior forceps.
2. stratum internum forceps.
3. „ externum.
4. pars inferior forceps.
5. stratum sagittale internum.
6. „ „ externum.

transverse cut through the stratum sagittale externum, as we know it from the healthy brain, is absent. Instead, we can appreciate a bright zone with roughly the same shape, which can be followed laterally to the convexity and medially also as a small bright stripe until the hippocampal gyrus (3). Under the microscope, only insignificant remnants of white matter can be seen within this zone. The stroke of the occipital lobe therefore caused a degeneration of the entire stratum sagittale externum in the temporal lobe. A marked contrast is the cingulum in the gyrus hippocampi, which usually joins the stratum sagittale externum and is now stained deep black (Plates 1–4).

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RESEARCH PAPER

White matter connections of the supplementary motor area in humans

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ABSTRACT

Introduction The supplementary motor area (SMA) is frequently involved by brain tumours (particularly WHO grade II gliomas). Surgery in this area can be followed by the 'SMA syndrome', characterised by contralateral akinesia and mutism. Knowledge of the connections of the SMA can provide new insights on the genesis of the SMA syndrome, and a better understanding of the challenges related to operating in this region.

Methods White matter connections of the SMA were studied with both postmortem dissection and advance diffusion imaging tractography. Postmortem dissections were performed according to the Klingler technique. 12 specimens were fixed in 10% formalin and frozen at -15°C for 2 weeks. After thawing, dissection was performed with blunt dissectors. For diffusion tractography, high-resolution diffusion imaging datasets from 10 adult healthy controls from the Human Connectome Project database were used. Whole brain tractography was performed using a spherical deconvolution approach.

Results Five main connections were identified in both postmortem dissections and tractography reconstructions: (1) U-fibres running in the precentral sulcus, connecting the precentral gyrus and the SMA; (2) U-fibres running in the cingulate sulcus, connecting the SMA with the cingulate gyrus; (3) frontal 'aslant' fascicle, directly connecting the SMA with the pars opercularis of the inferior frontal gyrus; (4) medial fibres connecting the SMA with the striatum; and (5) SMA callosal fibres. Good concordance was observed between postmortem dissections and diffusion tractography.

Conclusions The SMA shows a wide range of white matter connections with motor, language and limbic areas. Features of the SMA syndrome (akinesia and mutism) can be better understood on the basis of these findings.

INTRODUCTION

The supplementary motor area (SMA) is an eloquent region of the brain that plays an important role in the planning, initiation and learning of complex motor functions.^{1–4} The SMA in the dominant hemisphere is also involved in the initiation of speech.^{5,6} From a surgical perspective, the SMA is important as it is frequently involved by brain tumours; up to 10% of de novo glioblastomas and 27% of WHO grade II gliomas occur in this region.⁷ It is also a frequent surgical target for drug-resistant frontal lobe epilepsy.⁸ Surgery in this area can be followed by the 'SMA syndrome', as

originally described by Laplane,⁹ characterised by contralateral akinesia and, for surgery in the dominant hemisphere, mutism or speech reduction (also referred to as 'transcortical motor aphasia'). These symptoms tend to resolve over time, but can affect patients for several months and can occasionally be permanent.^{6,10,11} Knowledge of the connections of the SMA can help to understand the pathogenesis of the SMA syndrome, providing the neurosurgeon with a better understanding of the challenges related to operating in this region, as well as offering patients a more accurate preoperative counselling.

Although the connections of the SMA have been extensively studied in the primate with a detailed analysis of the connections of the pre-SMA and SMA-proper,^{12,13} limited data exist about the SMA connectivity in humans. The common method used to investigate the white matter in the primate is the autoradiography technique, where a neuronal tracer is injected in the area of interest and the connections of that particular area are then established with postmortem histology.¹⁴ In humans, two techniques are available to investigate white matter anatomy: *postmortem* dissection and diffusion tractography. Dissection of cadaveric specimens has been used for centuries to study the anatomy of the brain, including the tracts of white matter.¹⁵ In recent years, the technique originally described by Klingler¹⁶ has been revitalised in the neurosurgical community.¹⁷ A modification of this technique has been recently suggested, with the aim of sparing the cortex during the dissection (the so-called 'cortex-sparing' dissection).¹⁸ The advantage is maintaining the 3D relationship between the fibre tracts and their cortical terminations, thus avoiding the loss of fundamental landmarks during the dissection. Diffusor tractography is a relatively new MRI technique, and it is based on the principle that the diffusion of water molecules in white matter tracts is anisotropic, with the highest diffusivity occurring along the direction of fibres. Using mathematical models it is possible to estimate the one or more fibre orientations in each voxel and use this information to reconstruct the main white matter pathways with the unique advantage of investigating the white matter human anatomy in vivo.¹⁹

Diffusion tensor imaging and more advanced diffusion imaging methods have been used to study in humans the connectivity of the SMA region, showing fibres connecting the SMA with the fronto-opercular region (area 44 or 'Broca's area')

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and the striatum.^{20–23} However, there is no study in the literature that has comprehensively reviewed the white matter fibres connecting the SMA and no study using postmortem dissections has been dedicated to investigate this aspect.

In this study, for the first time we present a combined approach to investigate the SMA connections, with *postmortem* dissections and diffusion imaging performed *in vivo* on healthy volunteers. Original anatomical data about the SMA white matter connections are presented and discussed.

METHODS

Postmortem dissections

Postmortem dissection of white matter fibres was performed according to the technique originally described by Klingler.¹⁶ Twelve hemispheres in total were used for the present study (six right, six left). The hemispheres were collected at the Newcastle Brain Tissue Resource (Institute for Ageing and Health, Newcastle University, Newcastle upon Tyne, UK) and at the Department of Anatomy of Cantabria University (Santander, Spain). Both Institutions have ethical approval to use post-mortem human specimens for research purpose. The specimens were fixed in 10% formalin solution for a minimum of 3 months. After removal of the pia-arachnoid membrane and cortical vessels, the hemispheres were frozen at -15°C for 15 days. The water crystallisation induced by the freezing process disrupts the structure of the grey matter (which has a high water content), thus making it easier to peel off the cortex from the underlying white matter. The freezing process also separates the white matter fibres, facilitating the dissection of the tracts. The specimens were washed under running water for several hours before performing the dissection.

The superficial anatomy of each hemisphere was studied in detail, with identification of the sulci and gyri. The dissection was then started, with removal of the cortex and exposure of the underlying U-fibres (also known as intergyral or arcuate fibres). The white matter dissection was then completed in a stepwise manner, from lateral to medial and from medial to lateral. Wooden spatulas were used in the initial step of the dissection to carefully remove the cortex. Once the U-fibres were identified, the dissection was performed using blunt metallic dissectors with different tip sizes. Care was taken to separate the fibres using the blunt edge of the instrument, thus avoiding the generation of spurious tracts. In addition, dissection was performed according to the ‘cortex-sparing’ technique recently described by Martino *et al.*¹⁸ According to this technique, as much as possible of the cortex is preserved during the dissection in order to maintain the 3D relationship between the fibres and their cortical terminations. Digital images were acquired during the dissection.

MRI data

High-resolution diffusion imaging datasets from 10 adult healthy controls from the Human Connectome Project (HCP) database (<http://www.humanconnectome.org>, Release Q2) were used for this study.

Briefly, datasets were acquired with a voxel size of $1.25 \times 1.25 \times 1.25$ mm, field of view = 210×210 mm, 111 slices, TE = 89.50 ms, TR = 5520 ms, b-value = 1000, 2000, 3000 s/mm², 90 directions per b-values and 18 non-diffusion-weighted volumes. In this study, only data with a b-value of 2000 s/mm² were used. Data were preprocessed using the default HCP pre-processing pipeline (V2), which includes correction for susceptibility, motion and eddy current distortions. Spherical deconvolution was then performed using the damped

Richardson–Lucy deconvolution algorithm.²⁴ Algorithm parameters were $\alpha = 1.5$, algorithm iteration = 400 and $\eta = 0.06$ and $v = 8$ as regularisation terms. Spherical deconvolution tractography was performed using a modified Euler tractography algorithm²⁵ with a 45° angle threshold, an absolute hindrance modulated (HMOA) threshold of 0.012 and relative threshold 7%.

After whole brain tractography, semiautomatic dissections of tracts were performed according to predefined cortical and sub-cortical regions. Starting from an SMA region predefined on the Montreal Neurological Institute (MNI) space, non-linear transformations were calculated from MNI to each subject native space and applied on the SMA region. Other regions of interests were already available in the subject space as part of the HCP processing pipeline and derived from the structural data of each subject using freesurfer parcellation. Where required, exclusion regions of interest were manually placed to exclude remaining spurious or non-anatomically consistent streamlines. For each tract, binary maps were generated to identify voxels visited by at least one streamline and then averaged in the MNI space to generate visitation maps for each tract. In this study, we investigated for both hemisphere connections among the SMA and the inferior frontal gyrus (IFG), Caudate nucleus, precentral gyrus (PreCG) and cingulate gyrus.

Tract volume was used to estimate the lateralisation index (LI) for each tract using the following formula: $\text{LI} = (\text{Tract Volume left} - \text{Tract Volume right}) / ((\text{Tract Volume left} + \text{Tract Volume right}) / 2)$. Positive values indicate a left lateralisation. For each tract, a one sample t-test on the LI was performed using Bonferroni correction with an adjusted α level of 0.0125 per test (ie, 0.05/4). A Shapiro–Wilk test was used to verify normal distribution of LI in all tracts.

RESULTS

Each specimen was accurately examined and the major sulci and circonvolutions were identified. The central sulcus, dividing the PreCG and postcentral gyri, was the initial landmark to be identified on the lateral convexity. The precentral sulcus, anterior to the PreCG, was used as the posterior border of the SMA. The superior frontal sulcus, separating the superior frontal gyrus (SFG) and middle frontal gyrus (MFG), was considered as the infero-lateral border of the SMA.^{26 27} On the medial aspect of the brain, the cingulate sulcus, separating the mesial aspect of the SFG and the cingulate gyrus, represented the infero-medial border of the SMA. The anterior border was considered to be 5 cm in front of the precentral sulcus. The SMA is divided in pre-SMA and SMA-proper by a vertical line passing through the anterior commissure (vertical commissure anterior (VCA) line). On the medial aspect of each hemisphere, the VCA line was traced and the two regions of the SMA were identified (figure 1).

Dissection of the lateral aspect of the SMA

Short U-fibres

After removal of the cortex, the first fibres to be exposed on the convexity of the hemisphere were short arcuate fibres connecting neighbouring gyri (also known as ‘U-fibres’). In particular, U-fibres were encountered in the depth of the precentral sulcus, connecting the posterior part of the SMA-proper with the PreCG. The number and distribution of these fibres appeared to be variable, but a short arcuate fibre was always present in close proximity of the ‘hand knob’ of the primary motor cortex. This is a ‘knob-like’ portion of the PreCG corresponding to the middle knee of the central sulcus and representing the anatomical localisation of the motor hand area. Tractography showed consistently these connections bilaterally but without any

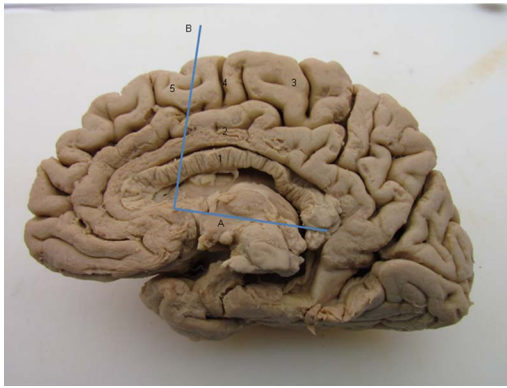


Figure 1 Medial aspect of a right hemisphere. The anterior commissure - posterior commissure (AC-PC) line is demonstrated (A). The VCA line (B) perpendicular to the AC-PC line at the level of the anterior commissure divides the pre-SMA anteriorly (5) from the SMA-proper (4). The corpus callosum (1), cingulate gyrus (2) and central lobule (3) are also indicated. SMA, supplementary motor area.

lateralisation ($LI = -0.13 \pm 0.34$, p value = 0.27). Similarly, U-fibres were encountered in the depth of the superior frontal sulcus, connecting the lateral aspect of the SMA with the MFG (figure 2, table 1).

Figure 2 (A) Lateral dissection of the SMA (right hemisphere). U-fibres are demonstrated in the depth of the precentral sulcus, connecting the PreCG and SMA-proper (red pins). U-fibres are also present in the superior frontal sulcus, connecting the SMA with the MFG (green pins). (1) PoCG; (2) PreCG; (3) SMA-proper; (4) SFG; and (5) MFG. (B) Diffusion tractography demonstrating the right and left connections of the SMA with the PreCG. Note the U-shaped fibres linking the two gyri. SMA, supplementary motor area; SFG, superior frontal gyrus; MFG, middle frontal gyrus; PreCG, precentral gyrus; PoCG, postcentral gyrus.

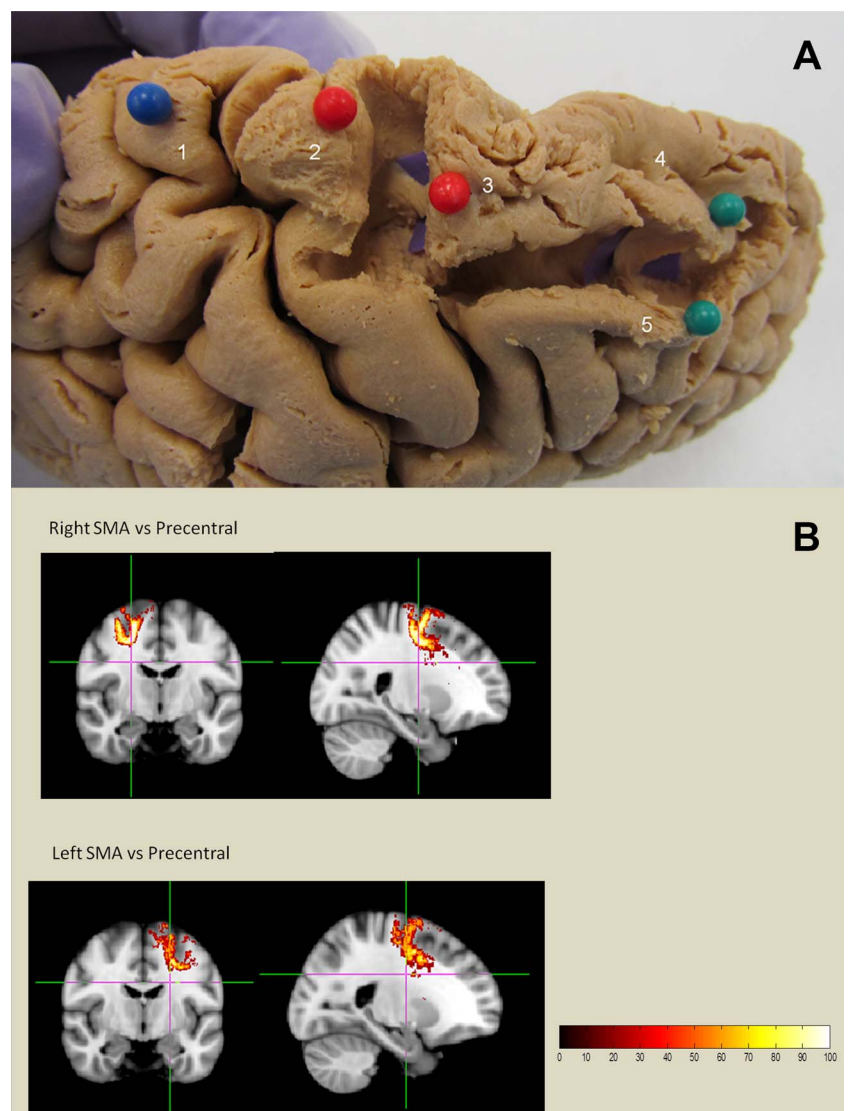
Frontal aslant tract

Continuing the dissection of the lateral aspect of the frontal lobe, a direct fibre tract running from the SFG to the pars opercularis of the IFG (area 44, according to Brodmann's classification) was demonstrated. After removal of the MFG and partial removal of the IFG, longitudinal fibres of the superior longitudinal fasciculus (SLF) were exposed. Deeper to the SLF, a vertical bundle of fibres, running from the SMA to the pars opercularis of the IFG, was observed. This fascicle presented an oblique course between the SFG and IFG, and corresponded to the frontal aslant tract (FAT). The cortical termination of this tract in the SFG was at the boundary between the pre-SMA and SMA-proper (figure 3). The tract was clearly demonstrated also with diffusion tractography where a large number of streamlines projected to the IFG pars opercularis. Fewer projections also reached the pars triangularis of the IFG and the inferior PreCG. The tract was always visible on both hemispheres and showed a statically significant left lateralisation ($LI = 0.38 \pm 0.31$, $p = 0.003$) (figure 3, table 1).

Dissection of the medial aspect of the SMA

Short U-fibres

On the medial aspect of the hemisphere, another set of U-fibres was observed, running in the depth of the cingulate sulcus. These fibres connected the SMA to the cingulate gyrus, running



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Table 1 Tract volume and lateralisation index (LI) for the diffusion tractography results

	Left volume (ml)	Right volume (ml)	Laterality index	p Value
Precentral gyrus	6.51±3.63	7.11±3.75	-0.13±0.34	0.270
Inferior frontal gyrus—pars opercularis	7.93±3.77	5.64±3.66	0.38±0.31	0.003
Cingulate gyrus—anterior	3.25±1.00	2.98±1.34	0.10±0.54	0.562
Caudate	2.01±1.95	1.38±2.22	0.45±0.69	0.105

Positive LI values indicate left lateralisation.

perpendicular to the cingulate sulcus. Diffusion tractography confirmed the presence of extensive and bilateral connections between the SMA and the anterior portion of the cingulate gyrus (figure 4). No significant lateralisation was observed with tractography (LI=0.10±0.54, p=0.56) (table 1).

Callosal fibres

Dissecting the mesial aspect of the brain, after removal of the cingulate gyrus, vertical fibres of the corpus callosum were exposed. These callosal fibres, clearly observed also with diffusion tractography, came from both the pre-SMA and the SMA-proper and converged on the rostrum and anterior part of the body of the corpus callosum (figure 5).

Corona radiata and striatal fibres

Continuing the dissection from medial to lateral, after complete removal of the cingulate gyrus and partial removal of the corpus callosum, the caudate nucleus was exposed. At this level, two sets of fibres running vertically from both the SMA-proper and pre-SMA were identified. The first group to be encountered belonged to the corona radiata and continued inferiorly, with a slight oblique course, into the internal capsule. More laterally,

another group of fibres converged directly onto the head and anterior body of the caudate nucleus. These fibres had a more vertical course and were situated more laterally to the callosal and corona radiata fibres. Diffusion tractography also showed connection between SMA and the head of the caudate nucleus (figure 6). However, the low number of streamlines and the rather disperse organisation of this tract produced a high variability in the volume of this tract. No significant lateralisation was observed (LI=0.45±0.69, p=0.10) (table 1).

DISCUSSION

To the best of our knowledge, the present study is the first in the literature dedicated to investigate the white matter connections of the SMA using both postmortem dissections (according to the traditional Klingler technique and the 'cortex-sparing' method) and advanced diffusion tractography. Overall, a good concordance was found between the two methods. All the connections described were identified with both techniques, with similar trajectories and directions of the fibre tracts observed. Postmortem dissections provided a more qualitative description of the fibres, being able to clearly identify the relationship of the fibres to other white matter tracts and adjacent cortical

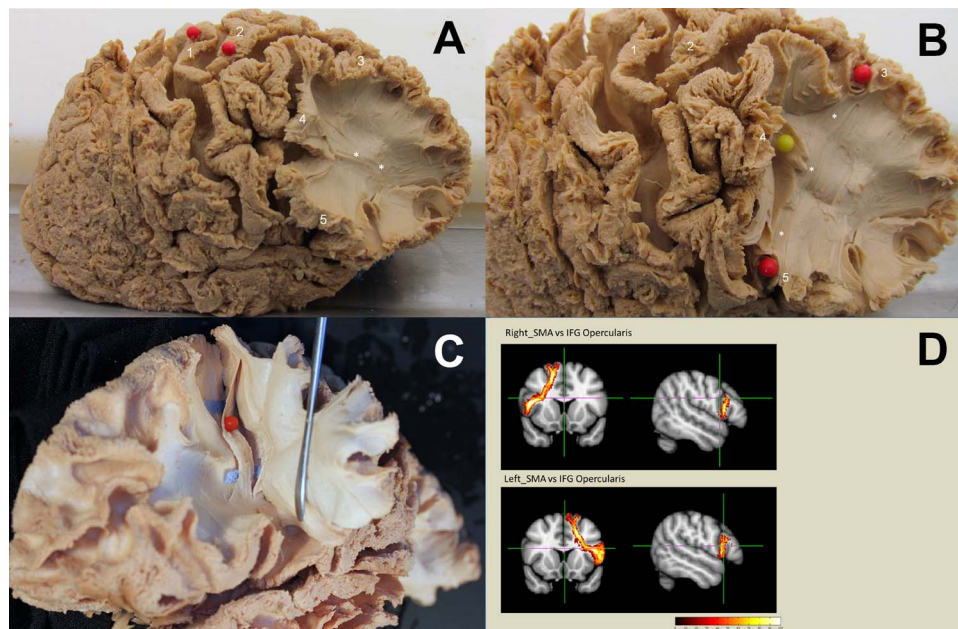


Figure 3 (A) Dissection of lateral aspect of the frontal lobe (right hemisphere): exposure of longitudinally-directed fibres of the SLF (asterisk) after removal of MFG. (1) PoCG; (2) PreCG; (3) SMA; (4) MFG (remnant portion); and (5) IFG. (B) Same specimen. The SLF has been divided (yellow pin), exposing the vertically oriented fibres of the FAT (asterisks), connecting the SMA with the pars opercularis of the IFG (red pins). (1) PoCG; (2) PreCG; (3) SMA; (4) MFG (remnant portion); and (5) IFG. (C) 'Cortex sparing' dissection of the left hemisphere. Exposure of the FAT (red pin). The MFG has been maintained and retracted by a blunt dissector. Note the cortical termination of the aslant tract at the level of the SMA and of the opercular portion of the IFG. (D) Diffusion tractography demonstrating the right and left FAT, connecting the SMA and the IFG. SMA, supplementary motor area; IFG, inferior frontal gyrus; MFG, middle frontal gyrus; SLF, superior longitudinal fasciculus; FAT, frontal aslant tract; PoCG, postcentral gyrus.

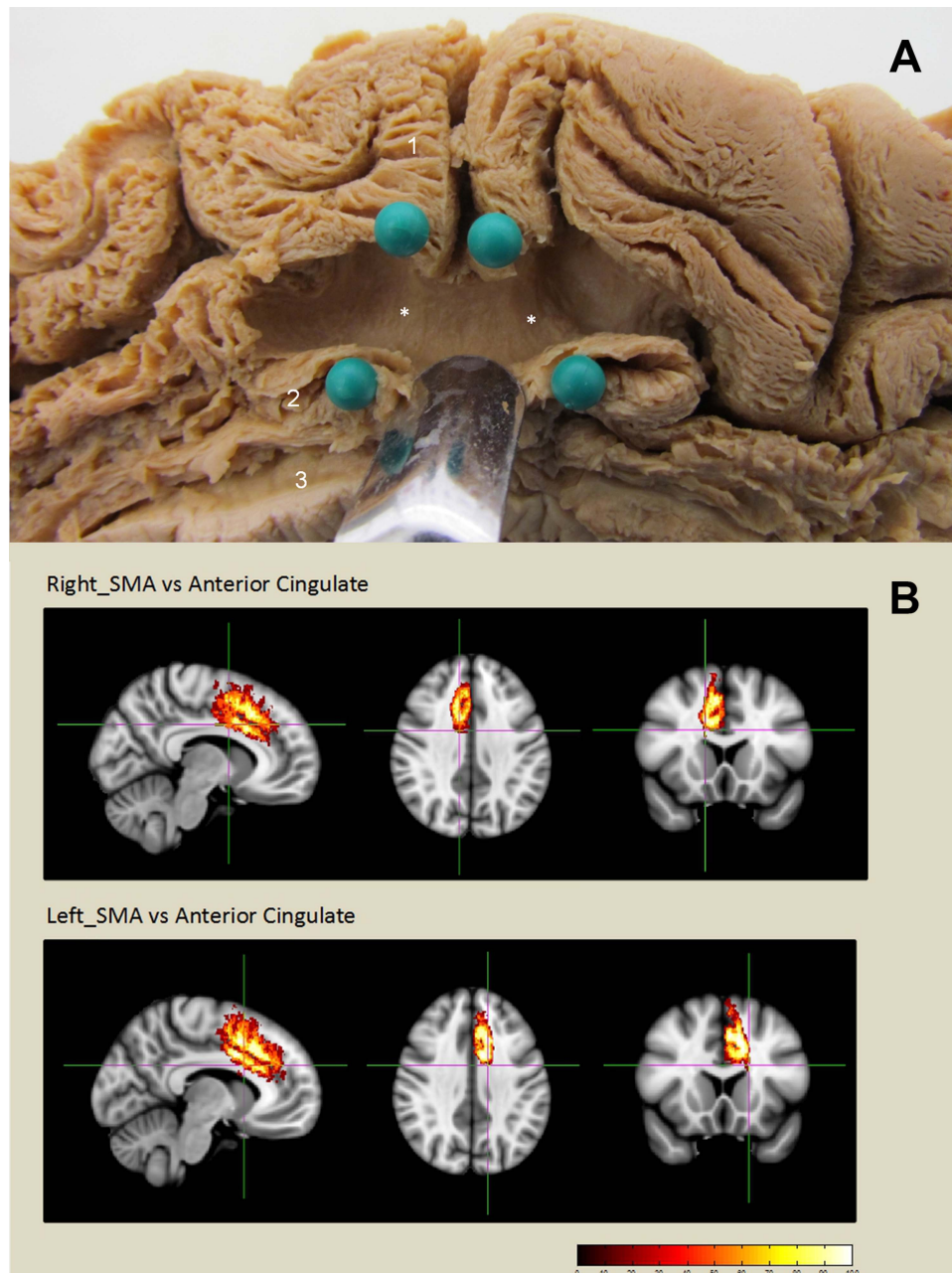


Figure 4 (A) Dissection of mesial aspect of the SMA (right hemisphere). U-fibers running in the cingulate sulcus, connecting the SMA with the cingulate gyrus (asterisks). The green pins show the cortical terminations of these U-fibers. (1) SMA; (2) cingulate gyrus; and (3) corpus callosum. (B) Diffusion tractography demonstrating the connections between the SMA and the cingulate gyrus. Note the extensive connections with U-fibers to the anterior portion of the cingulate gyrus. SMA, supplementary motor area.

landmarks. Tractography was particularly useful in adding quantitative analysis of tract volumes, making it possible to infer the lateralisation of the tracts described.

SMA and movement

Direct stimulation of the SMA induces a complex pattern of movements involving different muscles groups. Synergistic movements involving the head, the upper and lower limb (turning the head to look at the hand, grasping movements, etc) have been described.^{8 10 11 28} It is acknowledged that the SMA plays an important role in planning, initiation and execution of movements. In the primate, an SMA-proper and a pre-SMA have been differentiated on the basis of cytoarchitectonic and functional aspects.¹² Also in humans, in addition to the classic

SMA (SMA-proper), a more rostral area called 'pre-SMA' has been described. Neurophysiological studies suggest that the role of these two regions appear to be different with regard to motor function, with the pre-SMA being involved in higher motor control, such as planning and preparation of movement, while the SMA-proper is more directly related to the execution of movement.² In a recent paper by Ikeda *et al*,²⁹ epicortical field potentials were recorded with subdural electrodes from the pre-SMA and SMA-proper of epileptic patients. Their results showed that the pre-SMA is involved in cognitive motor control and decision making while the SMA-proper is more active in the initiation and execution of voluntary movements. The connections of the two regions can provide an anatomical basis for these functional aspects. It is known from the literature¹³ that

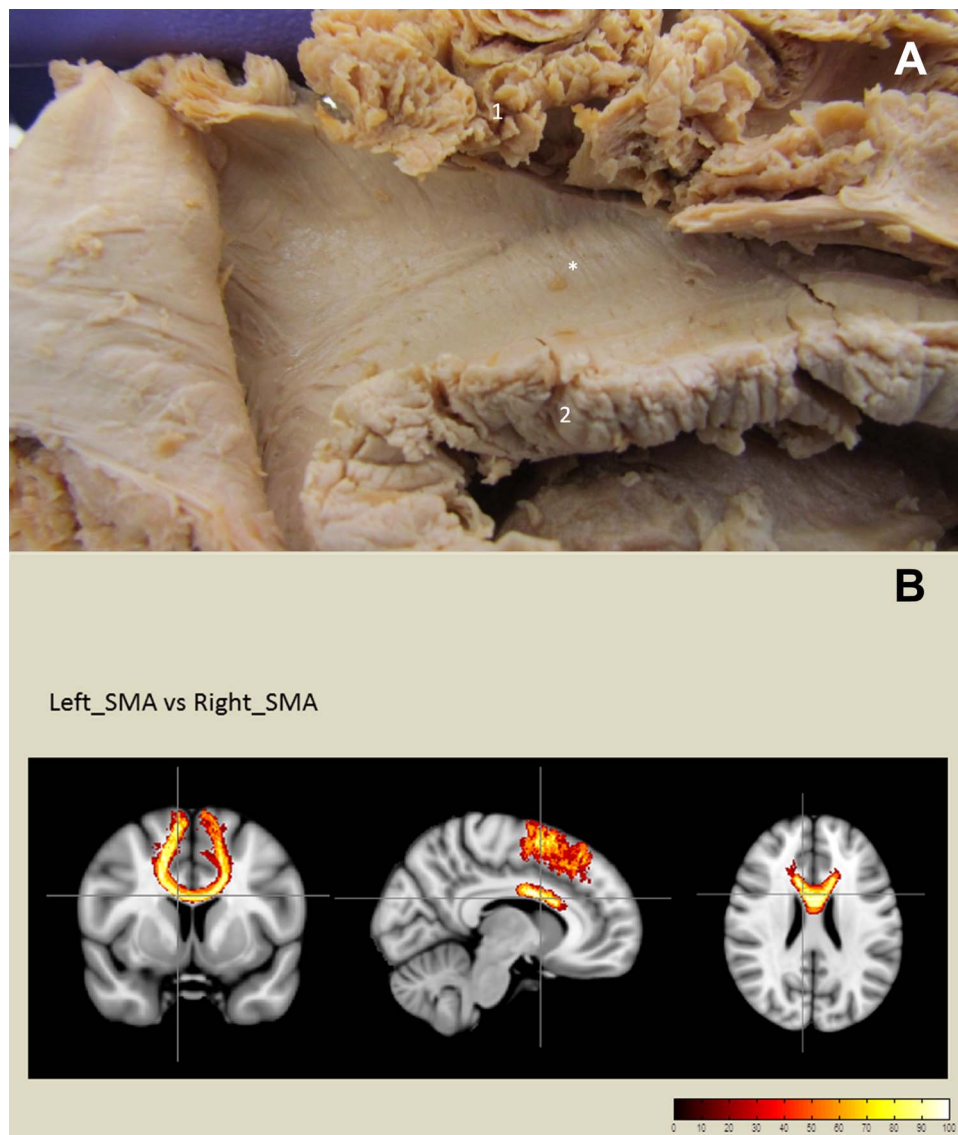


Figure 5 (A) Dissection of mesial aspect of the SMA (right hemisphere). Callosal fibres to the SMA observed after removal of the cingulate gyrus (asterisk). (1) SMA and (2) corpus callosum. (B) Diffusion tractography demonstrating the callosal fibres connecting the left and right SMA. SMA, supplementary motor area.

the SMA-proper sends fibres through the corona radiata and the internal capsule to join the cortico-spinal tract, while the pre-SMA has no direct connection with the spinal cord. Thanks to this cortico-spinal projection, the SMA-proper is thought to play a role in the direct execution of movement. In addition, our study has demonstrated the presence of short U-fibres in the depth of the precentral sulcus, directly connecting the SMA-proper with the primary motor cortex (M1). Of interest, a direct link between the SMA-proper and the M1 appears to be always present at the level of the hand region. It has been hypothesised that the convergence of fibres at this level can play a role in the control of complex hand movements, such as grasping and reaching.³⁰ Finally, both the SMA-proper and the pre-SMA have direct connection with the striatum, with fibres directed to the head and body of the caudate nucleus. The presence of striatal connections has been demonstrated in a DTI study by Lehericy *et al*,²⁰ and the results of our study are in accordance with these previous findings. This cortico-striatal connection is part of a wider network that, through the

thalamus, reverberates back to the cortex. This cortico-basal ganglia-thalamo-cortical network is implicated in different aspects of motor control, including initiation, sequencing and modulation of voluntary movements.³¹ The results of our diffusion tractography study suggest a predominance of these fibres on the left side in right-handed individuals.

SMA and language

Direct stimulation of the dominant SMA has been related to vocalisation and/or arrest of speech.^{8 28} Functional MRI studies have provided useful insights in the role of the SMA in language. The SMA appeared to be activated during different language tasks, such as repetition and silent verbal fluency, with the role of facilitating the initiation of speech.^{5 6 32} In the present study, we demonstrate the presence of a direct connection between the SMA and the pars opercularis of the IFG, corresponding to Broca's area in the dominant hemisphere. This tract has been called 'frontal aslant' (FAT) due to its oblique course.³⁰ Our results are in accordance with the previous description of

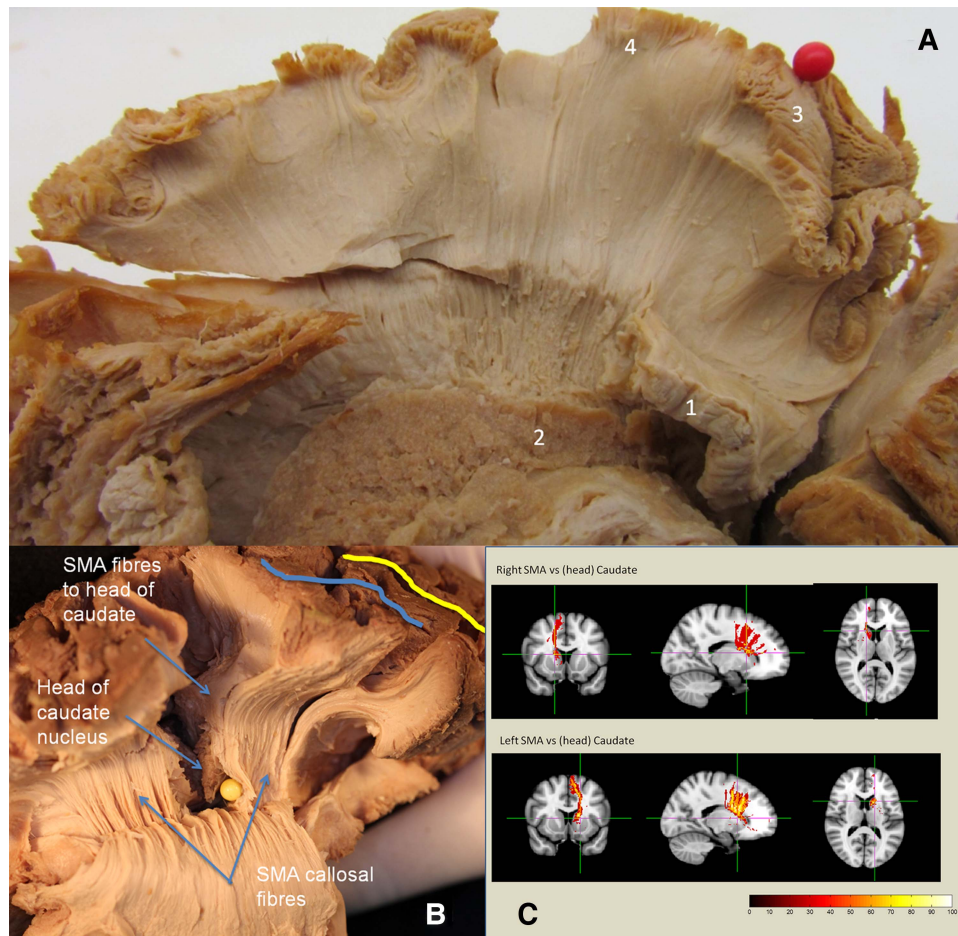


Figure 6 (A) Dissection of mesial aspect of the SMA (right hemisphere). Vertical fibres running from the SMA and converging on the caudate nucleus, seen after complete removal of the cingulate gyrus and of the corpus callosum. Red pin represents the PreCG. (1) Corpus callosum (remnant portion); (2) caudate nucleus; (3) PreCG; and (4) SMA. (B) 'Cortex sparing dissection' of a left hemisphere. The relationship between the SMA fibres to the head of caudate nucleus and the SMA callosal fibres is demonstrated. Yellow pin: ventricle. Blue line: precentral sulcus. Yellow line: central sulcus. (C) Diffusion tractography demonstrating the connections between the SMA and the caudate nucleus (right and left hemisphere). Note the convergence of the fibres onto the head of the caudate. SMA, supplementary motor area; PreCG, precentral gyrus.

the bundle provided by DTI studies.^{21–23} More recently, Kinoshita *et al*³³ have described a similar bundle connecting the medial frontal cortex and the IFG. The Klingler dissection performed by the authors suggests that the fibres of the FAT merge with the SLF. In our study, the FAT appears to be deeper than the SLF, which had to be cut in order to expose the underlying FAT. The SMA is thought to facilitate speech initiation due to the direct connection to the pars opercularis of the IFG provided by the FAT. In a study of the somatotopy of the SMA, Fontaine *et al*¹⁰ found that the language responses in the SMA were clustered at the border between the pre-SMA and SMA-proper. Interestingly, the border between pre-SMA and SMA-proper represents the cortical termination of the FAT, as demonstrated in the present study by both postmortem dissections and diffusion tractography. To further corroborate the role of the FAT in language, it has been reported that a damage to this tract is associated with decreased verbal fluency in primary progressive aphasia.³⁴ Finally, the left lateralisation of the FAT seen in our diffusion tractography, as previously reported by Catani *et al*,³⁰ is also consistent with a possible role in language played by this tract.

In the non-dominant hemisphere it has been suggested that the SMA may mediate voluntary control of face, tongue and pharynx movement through the FAT connecting the SMA and

frontal operculum. If the FAT is damaged, the information coming from the SMA cannot reach the oropharyngeal motor cortex, causing a selective volitional palsy as described in a case of Foix–Chavanié–Marie syndrome after removal of a right-sided fronto-insular low grade glioma.³⁵

SMA and limbic system

Studies from the primate have demonstrated extensive connections between the SMA and the limbic system, particularly through fibres directed to the cingulate gyrus.^{12–36} Our study confirms the presence of such connections in humans, where several U-fibres were visualised (both on dissection and diffusion tractography), running between the SMA and the cingulate. This direct link between motor and limbic areas is thought to play a role in the motor processing of negative emotional stimuli.³⁷ Oliveri *et al*³⁸ showed that conditioning transcranial magnetic stimulation of the SMA significantly increased the evoked motor potential recorded from the first interosseous muscle during visual-emotional triggered movements (the subjects had to perform movements in response to images with a negative emotional content). The SMA in humans may therefore interface the limbic and the motor systems in the transformation of emotional experiences into motor actions.³⁸ The fibres

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connecting the SMA and the cingulate gyrus are one anatomical substrate for this interaction.

White matter connections and the SMA syndrome

Surgery in the SMA can be followed by the 'SMA syndrome', originally described by Laplane in 1977.⁹ Reporting the results of the ablation of the SMA in three patients, Laplane described the clinical course after surgery as being characterised by global akinesia, more prominent contralaterally, with an arrest of speech. This was followed by a phase of recovery, when contralateral reduction of movement and severe reduction of speech were still present. This was followed by a complete recovery, when only a 'disturbance of the alternating movements of the hands'⁹ was present. Several surgical series related to tumours infiltrating the SMA have confirmed the original observations of Laplane,^{4 6 10 11 27} usually with more profound and marked symptoms following more extensive resection of the SMA, as it is the case with WHO grade II gliomas.³⁹ The outcome is usually favourable, with a complete recovery observed at 6–12 months after surgery. Minor symptoms, however, can be permanent (underutilisation of contralateral upper limb, mild word finding difficulties, etc).^{4 6 11}

The pathogenesis of the SMA syndrome is not fully understood. Our findings suggest that surgical ablation of the posterior SMA (SMA-proper) is more likely to induce a motor deficit, as this region is strongly connected with motor areas through fibres that directly join the cortico-spinal tract and U-fibres directed to the motor cortex. Specific motor symptoms, like deficit in grasping/reaching, can indeed be explained on the basis of the connection between SMA-proper and the hand region. The wide range of projections from the SMA to the striatum can provide the anatomical basis to explain the reduction in spontaneous movement, which is the hallmark of the SMA syndrome. Akinesia, a cardinal symptom of Parkinsonian disorders, can be the result of damage to the cortico-striatal network in which the SMA plays an important role in facilitating and sequencing voluntary movements. Finally, mutism and language reduction (transcortical motor aphasia), can be explained on the basis of the connection of the SMA with the Broca's area provided by the FAT. Indeed, some features of the classic Broca's aphasia are similar to the mutism observed in the SMA syndrome.

Knowledge of the anatomical connections of the SMA is relevant to the clinical implications of surgery in the SMA region. The degree of cortical and subcortical extension of SMA tumours (as assessed by preoperative MRI and DTI) can help to predict the postoperative course of patients, as previously demonstrated in the case of tumours growing in the ventrolateral frontal region.⁴⁰ This is important to offer patients a more informed counselling on the postoperative course of the SMA syndrome, as well as to plan in advance an individualised rehabilitative intervention.

CONCLUSIONS

The SMA is at the centre of a rich network of white matter connections with motor, language and limbic areas. The clinical aspects of the SMA syndrome, including akinesia and mutism, can be better understood on the basis of these anatomical findings.

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Anatomic Connections of the Subgenual Cingulate Region

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BACKGROUND: The subgenual cingulate gyrus (SCG) has been proposed as a target for deep brain stimulation (DBS) in neuropsychiatric disorders, mainly major depression. Despite promising clinical results, the mechanism of action of DBS in this region is poorly understood. Knowledge of the connections of the SCG can elucidate the network involved by DBS in this area and can help refine the targeting for DBS electrode placement.

OBJECTIVE: To investigate the anatomic connections of the SCG region.

METHODS: An anatomic study of the connections of the SCG was performed on postmortem specimens and in vivo with MR diffusion imaging tractography. Postmortem dissections were performed according to the Klingler technique. Specimens were fixed in 10% formalin and frozen at -15°C for 2 weeks. After thawing, dissection was performed with blunt dissectors. Whole brain tractography was performed using spherical deconvolution tractography.

RESULTS: Four main connections were found: (1) fibers of the cingulum, originating at the level of the SCG and terminating at the medial aspect of the temporal lobe (parahippocampal gyrus); (2) fibers running toward the base of the frontal lobe, connecting the SCG with frontopolar areas; (3) fibers running more laterally, converging onto the ventral striatum (nucleus accumbens); (4) fibers of the uncinate fasciculus, connecting the orbitofrontal with the anterior temporal region.

CONCLUSION: The SCG shows a wide range of white matter connections with limbic, prefrontal, and mesiotemporal areas. These findings can help to explain the role of the SCG in DBS for psychiatric disorders.

KEY WORDS: Accumbens frontal fascicle, Anorexia nervosa, Cingulum, Deep brain stimulation, Depression, Subgenual cingulate gyrus

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The subgenual cingulate gyrus (SCG) is the portion of cingulate cortex located beneath the genu of the corpus callosum, corresponding to Brodmann area 25. This region has gained considerable interest in recent years as a target for neuromodulation in neuropsychiatric disorders.¹ In particular, deep brain stimulation (DBS) of the white matter underlying the SCG has been proposed for treatment-resistant depression with promising results.^{2,3} More recently, DBS of the SCG also has been proposed for treatment-refractory anorexia nervosa, even if more clinical experience is needed to further validate this indication.⁴ It is thought that DBS can ameliorate the symptoms of depression by modulating the network of which SCG is a key

component.^{2,5,6} A detailed knowledge of the connections of the SCG can therefore help to elucidate the mechanism of DBS of the SCG. A better understanding of the anatomy of the white matter underlying the SCG also can assist the neurosurgeon in refining the positioning of the electrode within the SCG target.⁷

The “gold standard” technique to investigate the anatomy of white matter fibers is the tract tracing method used in animal experimental models. Different tracers can be injected into the cortex and are transported subsequently along the axons, providing labelling of white matter connections. Ex vivo specimens then are obtained from the animal brain, and the fibers are visualized under the microscope.⁸ More recent techniques use transgenic strategies to express fluorescent proteins in the nervous system. With this method, individual

ABBREVIATIONS: DBS, deep brain stimulation; SCG, subgenual cingulate gyrus

axons and multisynaptic networks can be described.⁹ The carbo-cyanine dye Dil has been used for tract tracing in postmortem human brains. Dil is strongly lipophilic and can diffuse bidirectionally along myelin sheaths in fixed specimens, allowing a long diffusion time. However, this technique is time-consuming, technically demanding, and relatively expensive.¹⁰ Two techniques are used commonly to investigate the white matter anatomy in humans. Postmortem dissections have been used for centuries to study the brain anatomy, including the tracts of white matter. In recent years, the technique originally described by Klingler¹¹ has been revitalized in the neurosurgical community.¹² This technique introduced the process of freezing previously fixed brains: the ice that forms between the fibers separates the white matter fascicles, thereby facilitating their dissection. In the past decade, several fiber dissection studies have been reported, providing original data and elucidating the anatomy of some controversial fascicles.¹³⁻¹⁵ The other method available to investigate the white matter anatomy is diffusion imaging. This is an MRI technique based on the principle that the diffusion of water molecules in white matter tracts is anisotropic. Tractography can reconstruct the direction of fiber tracts, with the unique advantage of investigating the white matter anatomy *in vivo*.^{16,17}

Limited data currently are available about the connections of the SCG, and these data are based on studies using diffusion imaging techniques only.¹⁸⁻²⁰ In the current anatomic study we adopted for the first time in the literature a combined approach to investigate the SCG connections, using both postmortem dissections and advance diffusion imaging. Original anatomic data about the SCG white matter connections are presented and discussed.

METHODS

Postmortem Dissections

Postmortem dissection of white matter fibers was performed according to the technique originally described by Klingler.¹¹ Ten hemispheres were used for the present study (5 right, 5 left). The specimens were fixed in 10% formalin solution for a minimum of 3 months. After removal of the pia-arachnoid membrane and cortical vessels, the hemispheres were frozen at -15°C for 15 days. The water crystallization induced by the freezing process disrupts the structure of the gray matter (which has a high water content), thus making it easier to peel off the cortex from the underlying white matter. The freezing process also separates the white matter fibers, facilitating the dissection of the tracts. After thawing, the specimens were washed under running water before the dissection was performed.

The superficial anatomy of each hemisphere was studied in detail, with identification of the sulci and gyri. On the medial aspect of each hemisphere, the corpus callosum was identified along with its constituent portions: rostrum, genu, body, and splenium. The cortex lying immediately under the genu was identified as the SCG. The dissection was started at this level. Wooden spatulas were used at the beginning to remove the cortex carefully. Once the underlying white matter fibers were exposed, the dissection was continued using blunt metallic dissectors with different tip sizes. Care was taken to separate the fibers using the blunt edge of the instrument, thus avoiding the generation of spurious tracts. The white matter dissection was completed in a stepwise manner, from medial to lateral. Digital images were acquired during the dissection.

Spherical Deconvolution Tractography

Twenty-two healthy male right-handed volunteers aged between 20 and 40 years were recruited. All subjects gave written consent. A semi-structured interview was used to exclude those subjects with a previous history of neurologic and psychiatric disorders. None of the participants were on medication.

For each participant, 60 contiguous near-axial slices were acquired on a 3T GE Signa HDx TwinSpeed system (General Electric, Milwaukee, Wisconsin) with the following parameters: rostro-caudal phase encoding, voxel size $2.4 \times 2.4 \times 2.4$ mm, matrix 128×128 , slices 60, NEX 1, echo time (TE) 93.4 ms, b-value 3000 s/mm^2 , 60 diffusion-weighted directions and 7 non-diffusion-weighted volumes, using a spin echo-echo planar imaging sequence. Peripheral cardiac gating was applied with effective repetition time (TR) of 20/30 R-R intervals. A sagittal 3-dimensional magnetization-prepared rapid acquired gradient echo dataset covering the whole head was also acquired (166 slices, voxel resolution = $1.2 \times 1 \times 1$ mm, TE = 2.8 ms, TR = 7 ms, flip angle = 8 degrees).

Diffusion datasets were corrected for head motion and eddy current distortions using affine registration to a non-diffusion-weighted reference volume as implemented in the FSL software package (FMRIB Analysis Group, Oxford, UK). White matter orientation estimation was performed using a spherical deconvolution approach able to estimate multiple orientations in voxels containing different populations of crossing fibers. Spherical deconvolution was applied using the damped Richardson-Lucy algorithm as described previously.²¹ The damped Richardson-Lucy algorithm reduces partial volume effects and spurious fiber orientations by providing reliable estimates of the fiber orientation distribution in voxels, which include mixed contributions of white matter, grey matter, and cerebrospinal fluid. Algorithm parameters were chosen with $\alpha = 1.5$, algorithm iteration = 200, and $\eta = 0.04$ and $\nu = 8$ as regularization terms.²¹ Whole-brain spherical deconvolution tractography was performed, selecting every brain voxel with at least one fiber orientation as a seed. Streamlines were propagated using a modified Euler tractography algorithm²² with an absolute hindrance modulated orientational anisotropy threshold of 0.015, a relative threshold 7%, and 60 degrees as the angle threshold. After whole brain tractography, a novel semiautomatic approach, named MegaTrack, was used to dissect all tracts from the 22 subjects.²³ Briefly, all streamlines from each dataset are first nonlinearly registered and remapped to the standard Montreal Neurological Institute space and concatenated to create a single "mega" tractography dataset containing the streamlines from all subjects. A single manual dissection of this dataset allows the simultaneous dissection of all subjects' anatomy. Multiple inclusion and exclusion regions are used to dissect bundles of interest and remove spurious and anatomically inconsistent streamlines. Individual anatomy and tract-specific measurements from each subject then can be obtained by recovering predefined subject and streamline IDs from the final dissected tracts. The software tools for spherical deconvolution tractography and MegaTrack analysis were developed using Matlab (<http://www.mathworks.com>).

A lateralization index (LI) of the volume of the tracts identified was calculated using the following formula: $2 \times (\text{Vol Left} - \text{Vol Right}) / (\text{Vol Left} + \text{Vol Right})$. Positive values indicate a left lateralization (Table). For each tract, a one-sample *t* test on the LI was performed. Non-significant lateralization was observed with and without Bonferroni correction.

RESULTS

The SCG was identified on the medial aspect of each hemisphere. The cingulate gyrus was in direct continuation with the

TABLE. Results of Volume Calculation and Lateralization Index for Each Tract Described

	Left Volume, mL	Right Volume, mL	Lateralization
Cingulum	24.36 ± 3.06	22.958 ± 3.20	0.060 ± 0.130
Uncinate	11.90 ± 4.29	11.949 ± 2.65	-0.051 ± 0.328
Frontoaccumbens	2.51 ± 0.77	2.870 ± 1.26	-0.087 ± 0.444
Frontopolar	1.61 ± 0.94	1.35 ± 0.99	0.053 ± 0.799

SCG, after the contour of the corpus callosum. The sulcus of the corpus callosum divided the cingulate gyrus (superiorly) and the corpus callosum (inferiorly). The cingulate sulcus divided the cingulate gyrus (inferiorly) from the mesial aspect of the superior frontal gyrus (superiorly). At its inferior edge, the cingulate sulcus divided the SCG (posteriorly) from the paraolfactory gyrus anteriorly. Posteriorly, the SCG was bordered by the rostrum of the corpus callosum and the anterior commissure (Figure 1).

Cingulum

The first fibers encountered dissecting the SCG cortex were fibers belonging to the cingulum. Completing the removal of the cingulate gyrus, the cingulum was thus progressively exposed. It appeared as a C-shaped tract running deep to the cingulate gyrus and developing around the corpus callosum. From its most anterior aspect, at the level of the SCG, this tract runs longitudinally, passing anteriorly to the genu and superiorly to the body of the corpus callosum. Posteriorly to the splenium, at the level of the isthmus of the cingulate gyrus, the tract narrows, taking an anterior course and extending into the mesial aspect of the temporal lobe,



FIGURE 1. Mesial surface of a right hemisphere prepared according to the Klingler technique. 1: rostrum; 2: genu; 3: body; 4: splenium of corpus callosum; 5: SCG; 6: cingulate gyrus; 7: frontopolar region; 8: gyrus rectus.

where it terminates at the anterior portion of the parahippocampal gyrus, adjacent to the hippocampus. Distinct groups of superficial vertical fibers were observed running between the cingulum and adjacent gyri. Anteriorly, small vertical fibers to the superior frontal gyrus and to the paracentral lobule were identified. More posteriorly, a larger and more easily identifiable group of vertical fibers was directed between the cingulate and the precuneus (Figures 2 and 3). This tract did not show a significant lateralization (LI: 0.060 ± 0.130 , $P > .05$).

Frontopolar Connections

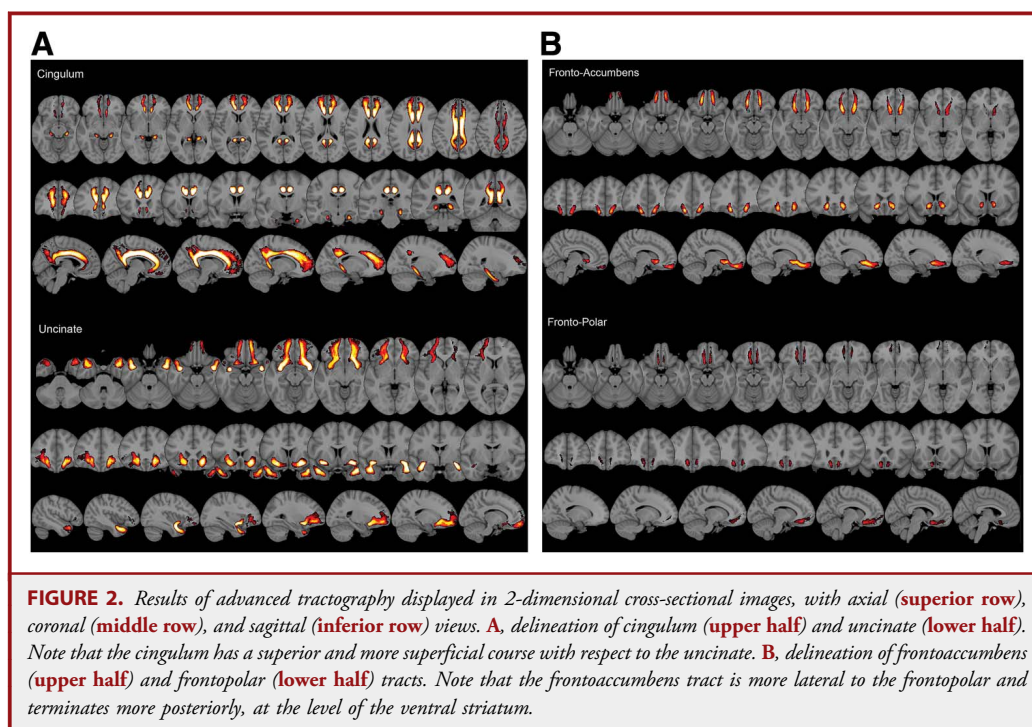
A group of fibers was observed running from the SCG anteriorly, toward the basal and mesial aspect of the frontal pole. Inferiorly, after removing the cortex of the paraolfactory gyrus, a short longitudinal tract was exposed. This tract runs between the SCG and the medial aspect of the frontal pole (corresponding to Brodmann areas 11 and 12). More superiorly, other fibers were observed between the SCG and the inferior portion of the medial frontal gyrus (medial part of the superior frontal gyrus), corresponding to Brodmann area 32. These prefrontal connections appeared to converge posteriorly toward the SCG, almost blending with the posterior portion of the cingulum (Figures 2 and 3). This tract did not show a significant lateralization (LI: 0.053 ± 0.799 , $P > .05$).

Uncinate Fasciculus

Dissection of the inferior portion of the medial frontal lobe, with removal of the paraolfactory gyrus and partial removal of the rectus gyrus, exposed fibers running posteriorly and inferiorly with respect to the SCG and to the frontopolar fibers previously described. These fibers have a hook-shaped course, curving anteriorly to the ventral portion of the striatum to form the mesial part of the temporal stem. The fibers continue their course until reaching the most anterior aspect of the mesial temporal pole, adjacent to the amygdala. These fibers correspond to the mesial portion of the uncinate fasciculus, which runs from the SCG and gyrus rectus to the mesial temporal pole (Figures 2 and 4). This tract did not show a significant lateralization (LI: -0.051 ± 0.328 , $P > .05$).

Callosal and Striatal Fibers

Continuing the dissection in a medial direction, after complete removal of the cingulum and prefrontal fibers, the deeper fibers of the corpus callosum were exposed. These fibers have a fan-like appearance and run anteriorly and laterally, contributing to the forceps minor. At the most inferior edge of the dissection, deep to the rectus gyrus, a small bundle was observed running from the frontal pole in a posterior direction, converging onto the ventral portion of the striatum (at the site of the nucleus accumbens). This bundle corresponds to the previously described accumbofrontal fascicle.²⁴ It runs through the white matter of the SCG, in a plane lateral to the frontopolar connections and superiorly to the medial part of the uncinate fasciculus, and terminates at the level of the



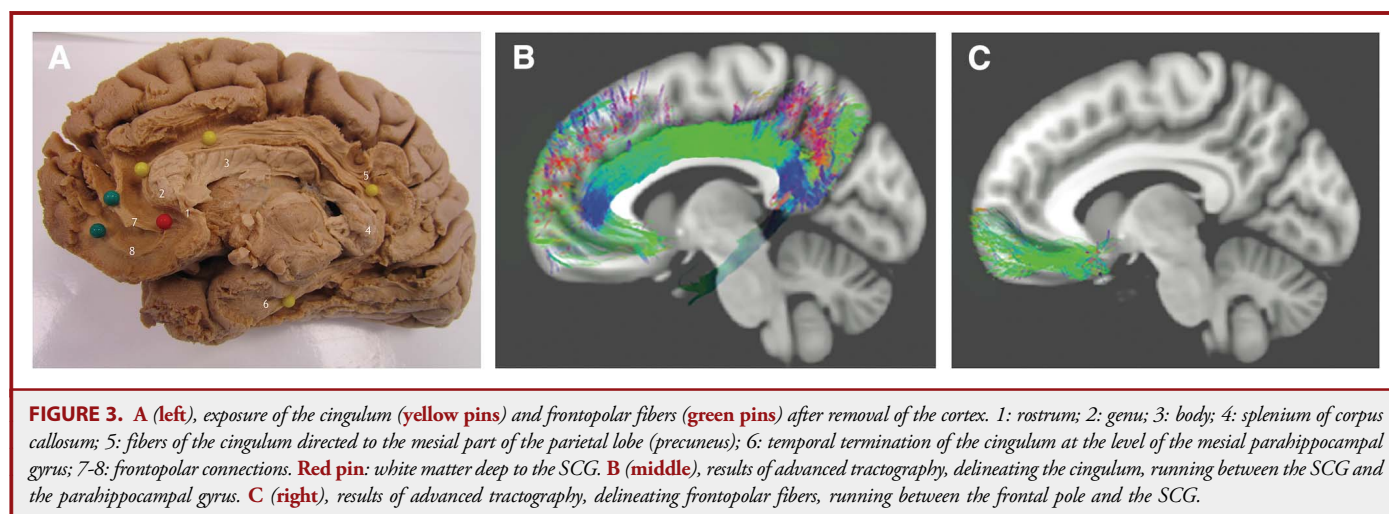
ventral striatum (Figures 2 and 5). This tract did not show a significant lateralization (LI: -0.087 ± 0.444 , $P > .05$).

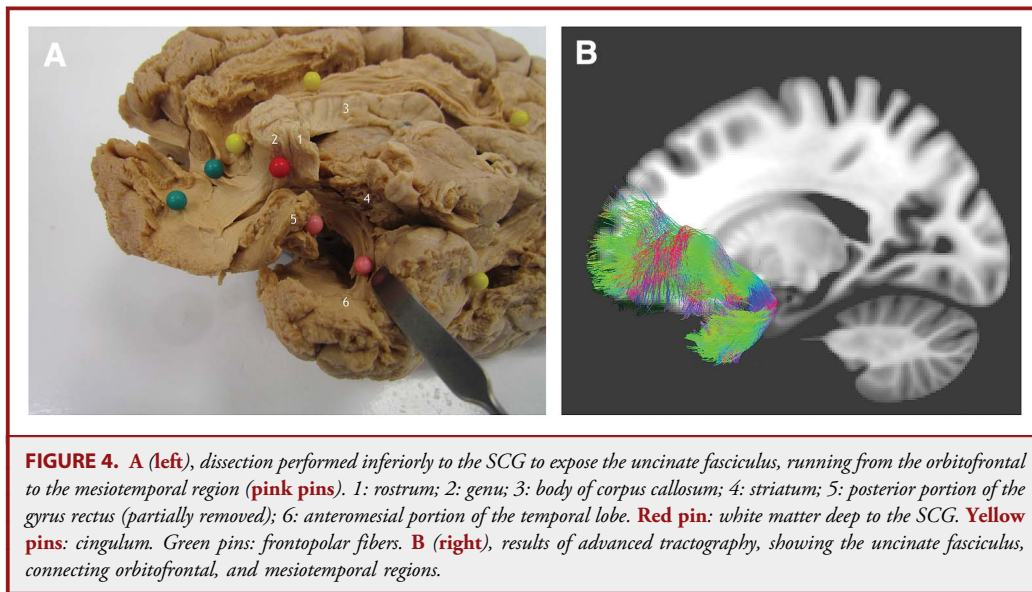
DISCUSSION

White Matter Connections of SCG

In the present article, we present a comprehensive description of the connections of the SCG. This area appears to be at the center of a rich network of fibers, connecting the SCG to the cingulate, hippocampus, amygdala, orbitofrontal cortex, and ventral striatum. To the best of our knowledge, this is the first time that these

connections have been investigated using a combined approach, using both postmortem dissections and advance diffusion imaging. Previous studies used probabilistic tractography to investigate the connections of the SCG in humans.^{18,19} The results of these studies demonstrated an overall similar pattern of connections, with projections to the nucleus accumbens, amygdala, hypothalamus, and orbitofrontal cortex. In our study, connections to the amygdala and the anterior part of the temporal lobe appeared to be mediated via the mesial portion of the uncinate fasciculus, while connections to the hippocampus are mediated via the cingulum. Similarly, we identified short intralobar frontal fibers directed to the frontopolar

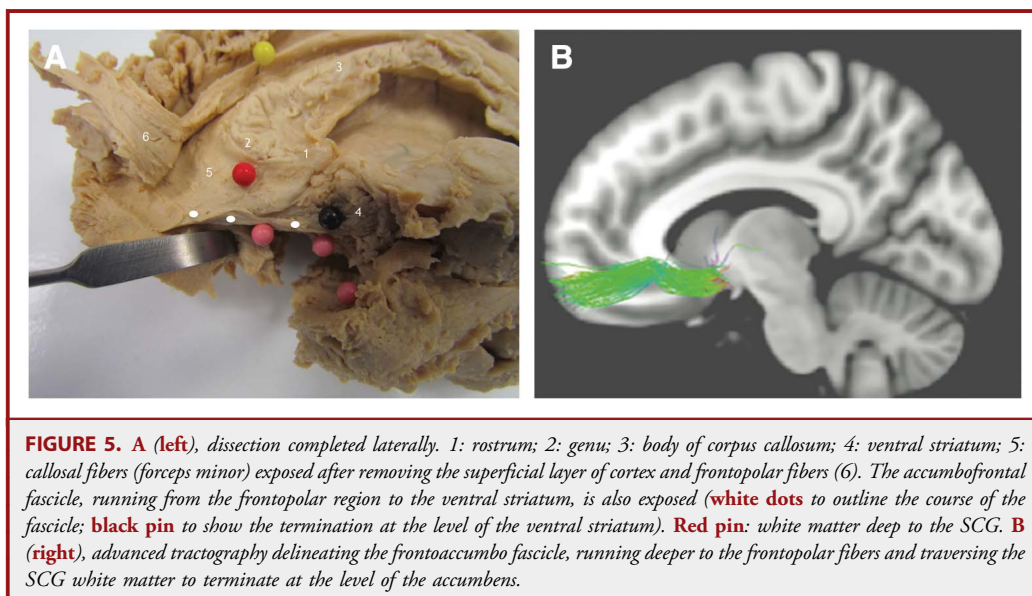




cortex. In our study, we did not observe a direct connection to the hypothalamus, whereas fine fibers were seen converging onto the ventral portion of the striatum/nucleus accumbens. It has to be noted that probabilistic tractography has some methodologic limitations.¹⁸ ROI sizes, along with the geometrical property (size and dimension) of the bundles studied can affect the final results of tractography, introducing false positive or false negative results. Our approach has the merit of combining the results of a traditional anatomic technique, the Klingler dissection, with advance diffusion tractography. One caveat of the Klingler method is to avoid creating false tracts when performing the dissection,¹⁴ while a limit is the difficulty in obtaining volume comparison of single tracts

between hemispheres (in the same subject) and between different subjects. It has been demonstrated previously by our group that the 2 techniques can be used to validate each other with a good concordance observed between the 2.^{25,26} All the SCG connections described in the present article were identified with both techniques, with similar trajectories and directions of the fiber tracts observed. In addition, diffusion data allowed for the assessment of the lateralization of the tracts described.

The first, large connection to the SCG identified was the cingulum. This fascicle has been extensively studied in primates and in humans. These studies demonstrated that the cingulum contains fibers from the cingulate cortex to isocortical areas



(high-order association areas in the frontal and parietal cortices) and to paralimbic and limbic cortices (parahippocampal gyrus and entorhinal cortex at its temporal termination). Our anatomic study confirms the presence of extensive U-fiber connections between the cingulum and mesial frontal and parietal cortex, along with projections to the parahippocampal region. James Papez²⁷ was the first to stress the importance of the cingulum in describing an anatomic circuit for the processing of emotions. In Papez's limbic circuit, the cingulum represents the external ring, connecting the cingulate gyrus with the hippocampus, while the fornix represents the internal ring, connecting the hippocampus with the mammillary bodies, which are eventually connected to the anterior thalamus via the mamilothalamic tract of Vicq d'Azir and from here back to the cingulate gyrus. From the initial observations of Papez, the fiber system of the cingulum has been implicated in a variety of emotional and behavioral responses that may play a role in depression and anorexia nervosa. Complex motor behaviors, emotional coloring of sensation and nociception, and avoidance behavior have been linked to the anterior cingulate region and cingulum bundle.⁸ Modern studies also have shown an abnormality in fractional anisotropy of the cingulum in patients affected by psychiatric disorders, such as schizophrenia,²⁸ obsessive compulsive disorder,²⁹ and major depression.³⁰ Of interest, the largest U-fibers connection identified in the present study was between the cingulum and the mesial aspect of the superior parietal lobe (precuneus). In anorexia nervosa, projection to the parietal lobe and parietal lobe dysfunction have been implicated in the generation of an altered body representation, which is one of the main features of the disease.³¹ In summary, the cingulum bundle appears to be an essential "dorsal" limbic pathway connecting the frontal, parietal, cingulate, and ventral temporal cortices.⁸

The second large associative bundle identified in our study and intimately related to the SCG region is the uncinate fasciculus, connecting the rostral temporal regions with the medial and orbital cortices. Our results are in accordance with previous description of this bundle, which runs in the anterior third of the temporal stem.¹⁴ This tract has been considered as the essential component of a "ventral" limbic pathway.⁸ The anterior temporal lobe is involved in processing modality-specific information, such as auditory (rostral superotemporal gyrus), visual (rostral inferotemporal region), gustatory (rostral insular opercular cortex), and emotional (amygdala) information.³² The orbitofrontal cortex seems to be involved in the emotional response to these stimuli,³³ along with self-regulation and decision-making.³⁴ The uncinate fasciculus therefore connects temporal areas that contain modality-specific and multimodal information with frontal areas that regulate behavior and emotional response to these stimuli.

A third group of fibers described in our dissections are directed from the SCG toward medial prefrontal areas, including Brodmann areas 11, 12, and 32. Studies on the primate demonstrated that neurons in the orbitofrontal cortex encode economic value, playing a major role in value assignment underlying economic choices.³⁵ Functional neuroimaging studies in humans similarly

have implicated these areas in detection of pleasant and unpleasant emotions and reward-based decision making.^{36,37} This last aspect in particular has been associated with addiction behavior, and the medial prefrontal cortex has been proposed as a target for DBS in addiction.³⁸ Of interest, an alteration in reward-based decision making and addiction behavior have been described in both major depression and anorexia nervosa.

A small contingent of fibers also has been observed in the present study between the frontopolar area and the ventral striatum, seat of the nucleus accumbens. The nucleus accumbens has been considered to play a central role in the reward circuitry, acting as an interface between limbic and motor systems.³⁹ fMRI studies have shown repeatedly that receipt of rewards increases blood oxygen level dependent responses in the nucleus accumbens.⁴⁰ The nucleus accumbens has been implicated in circuitry of anorexia nervosa and depression, in which an alteration in the perception of reward is a common feature.⁶ DBS of the nucleus accumbens has been proposed for a number of psychiatric conditions, including addiction, Tourette syndrome, and depression.^{1,24}

SCG White Matter and DBS for Neuropsychiatric Disorders

In recent years, the development of DBS, initially for the management of movement disorders, has introduced the possibility of neuromodulation in the field of psychosurgery, spurring a new interest in this field of functional neurosurgery. DBS has been proposed with success by different groups for the treatment of obsessive-compulsive disorders, with the subthalamic nucleus, nucleus accumbens, and anterior limb of the internal capsule identified as potential targets.¹ More recently, DBS of the SCG has been introduced for treatment-resistant depression and proposed for anorexia nervosa (although more experience will be required to validate anorexia nervosa as an indication for DBS). The SCG has been selected as a target for these psychiatric disorders on the basis of functional neuroimaging studies that demonstrated an altered metabolism in this region in affected patients. Metabolic studies with positron emission tomographic scans showed a decrease in glucose metabolism in patients receiving successful pharmacologic treatment for depression.^{41,42} The advantage of DBS vs lesioning is that DBS can reversibly and adjustably modulate the activity of the structures involved, whether these are grey matter or white matter structures. In the context of movement disorders, stimulation of white matter fibers of the zona incerta and Forel fields has been proposed to explain the clinical efficacy of DBS of the subthalamic region in ameliorating the symptoms of Parkinson disease.⁴³ In the same way, we speculate that stimulation of the white matter fibers of the SCG can play a role in controlling symptoms of depression and anorexia nervosa by modulating the network involved by SCG connections.^{2,5,6}

The results of our study show that the SCG white matter is at the center of a network involving limbic, prefrontal, and mesiotemporal areas. This information is of potential clinical interest in refining the targeting for SCG DBS. A lesion analysis for

limbic leucotomy showed that the structures lesioned in patients who responded favorably to limbic leucotomy correspond largely to the SCG connections described in the present article, including the cingulum, the uncinate fasciculus, the nucleus accumbens, the medial orbitofrontal cortex, and the amygdala.⁴⁴ In a previous multicenter study of subcallosal area DBS for treatment-resistant depression, a minimal variability in the location of the active contacts in the white matter deep to the subgenual gyrus has been reported among participating centers.⁴⁵ This suggests that stimulation of a relatively small area of white matter at the crossroad of all the connections described in the present article can contribute to the clinical effect observed in patients. This also may explain the need for the relatively higher stimulating voltages to reach clinical effect in treatment-resistant depression, compared with those typically used in movement disorders in which the target is more anatomically confined and well defined. A more recent study used probabilistic tractography to delineate the white matter pathways mediating successful DBS of the SCG in patients with treatment-resistant depression.⁴⁶ The results of this study again show a large correspondence with the findings of the present investigation, with the cingulum, uncinate fascicles, and forceps minor playing a role in mediating DBS for treatment-resistant depression. In addition, our study also showed the presence of connections to the nucleus accumbens, which has also been suggested as a target in treatment-resistant depression.²⁴

It therefore seems possible to conclude that modulation of these fibers can contribute to the clinical benefit observed after DBS of the SCG. Knowledge of the white matter connections deep to the SCG, as assessed on preoperative diffusion tractography, can help in guiding the implantation of the DBS electrodes in the individual patient. In a recent study, targets within the SCG selected using diffusion imaging seemed to be significantly different in location from those selected using conventional T2 sequences. This was considered to have the potential to enhance treatment outcome by reducing the impact of interindividual variability.⁷ As exemplified in our work (Figure 5), a reconstruction of the white matter fibers running through the SCG region of the individual patient can be incorporated in the surgical planning of DBS to improve lead location.

Results of the lateralization analysis demonstrated that no difference was found between left and right hemispheres in the tracts described, ie, the tracts of the subgenual region are equally represented in the 2 hemispheres. This can be consistent with the notion that surgery for mood disorders involving the white matter of the subgenual region need to be performed bilaterally to be successful clinically. Early studies involving limbic leukotomy and subcaudate tractotomy reported that the clinical effect was observed only after bilateral lesions were performed.¹ These early clinical observations, along with the anatomic findings provided in the present study, support the contemporary practice in DBS for mood disorders, in which SCG electrodes are implanted bilaterally.^{3,45}

CONCLUSION

The white matter fibers deep to the SCG are at the center of a large network, connecting prefrontal, limbic, and mesotemporal regions. Two large associative bundles related to the limbic system, the cingulum, and the uncinate fasciculus, are part of the SCG network. Short intralobar fibers connect the SCG to the frontopolar region and the frontopolar region to the accumbens. Comprehensive knowledge of the anatomy of these fibers can help explain the clinical effect of neuromodulation in neuropsychiatric disorders such as depression and anorexia nervosa.

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Acknowledgments

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APPENDIX B

(previous work recognised for doctoral submission)

A lateralized brain network for visuospatial attention

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Declan G M Murphy¹ & Marco Catani^{1,3}

Right hemisphere dominance for visuospatial attention is characteristic of most humans, but its anatomical basis remains unknown. We report the first evidence in humans for a larger parieto-frontal network in the right than left hemisphere, and a significant correlation between the degree of anatomical lateralization and asymmetry of performance on visuospatial tasks. Our results suggest that hemispheric specialization is associated with an unbalanced speed of visuospatial processing.

Clinical studies in patients with brain lesions and decades of neuropsychological testing in split-brain patients have led to the assumption that visuospatial attention is a function of the right hemisphere^{1,2}. Nevertheless, right visuospatial neglect is a frequent finding in patients with left hemisphere damage³ suggesting that visuospatial attention is probably a bilateral function, with right hemisphere dominance in most, but not all, humans⁴. The anatomical basis of the hemispheric dominance for visuospatial attention is largely unknown.

In the monkey brain, the activity of neurons dedicated to visuospatial attention has been recorded simultaneously using multiple electrodes implanted in the parietal and frontal cortices⁵. Axonal tracing studies have shown that these neurons are directly linked through a system of connections running longitudinally in the dorsolateral regions of the brain⁶. Recent structural⁷ and functional⁸ imaging studies provide indirect evidence of a similar system in the human brain. However, the hemispheric lateralization of the parieto-frontal connections in the human brain and its correlation with visuospatial performances has never to our knowledge been demonstrated.

In this study, we performed virtual *in vivo* dissections of the parieto-frontal connections in 20 right-handed subjects (11 males, 9 females) using diffusion imaging tractography based on spherical deconvolution⁹. A comparison between our human tractography dissections and the corresponding reconstructions from a monkey atlas⁶ that we modified is illustrated in **Figure 1**. Overall, parieto-frontal connections of the human and the monkey brain are similarly organized in three longitudinal parieto-frontal tracts separated into a dorsal superior longitudinal fasciculus (SLF) I, middle SLF II and ventral SLF III. These findings were confirmed with post-mortem dissections in one human

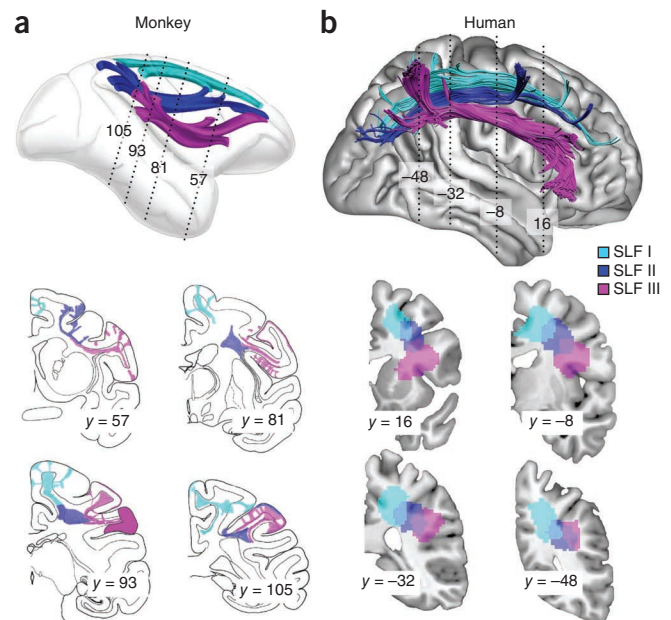


Figure 1 The three branches of the superior longitudinal fasciculus (SLF I, II and III). (a,b) Comparison between axonal tracing in monkey^{6,10} (a) and *in vivo* spherical deconvolution (SD) tractography in humans (b). Three-dimensional reconstructions are displayed at the top of each panel, and coronal sections at the indicated y planes are at the bottom.

right hemisphere (**Supplementary Methods, Supplementary Results, Supplementary Note, Supplementary Figs. 1–7 and Supplementary Tables 1 and 2**)¹⁰.

By measuring the volumes of the tracts in both hemispheres we were able to show a dorsal to ventral gradient of lateralization of the SLF (**Fig. 2a**). The SLF I is symmetrically distributed between left and right hemispheres ($t_{19} < 1$); the SLF II shows a trend of right lateralization ($t_{19} = 1.141$; $P = 0.268$) and the SLF III is right lateralized ($t_{19} = 6.083$; $P < 0.001$).

To test whether this lateralization was related to the attentional bias for one visual hemifield, we asked the participants to perform a line bisection test¹¹. In the general population, a small left deviation in the line bisection test is observed and referred to as the ‘pseudoneglect effect’¹¹. Consistent with previous studies¹², our participants deviated toward the left at a group level (-1.53 ± 0.02 mm; $t_{19} = 3.148$; $P = 0.005$). In most participants, the correlation analysis indicates that larger SLF II volumes on the right hemisphere corresponded to a greater deviation to the left in the line bisection ($r = -0.734$; $P < 0.001$) (**Fig. 2b**). Notably, the three subjects deviating to the right showed

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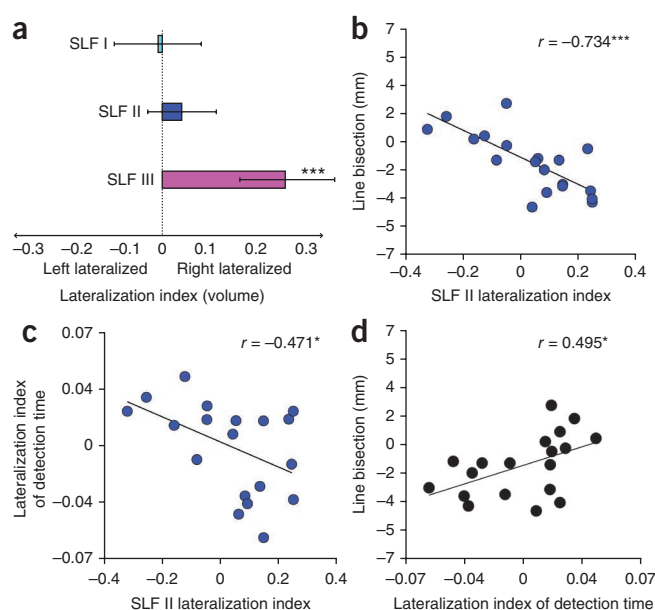


Figure 2 Correlations between anatomical and behavioral lateralizations. (a) Hemispheric lateralization of the three SLF branches, with 95% confidence intervals. (b,c) Correlations between the lateralization of the SLF II and both the deviation on the line bisection task (b) and the lateralization of the detection time (c). (d) Correlation between the deviation on the line bisection task and the detection time. $^*P < 0.05$ and $^{***}P < 0.001$.

an opposite pattern of lateralization (that is, larger volume of the left SLF II; see **Supplementary Table 1**). Correlations with the SLF I ($r = 0.258$; $P = 0.286$) and the SLF III ($r = -0.295$; $P = 0.220$) were not statistically significant.

It is unknown how differences between the two hemispheres in SLF II volume can lead to asymmetrical processing of visual scenes. A larger tract in the right hemisphere could depend on several factors, including greater fiber myelination, more axons and larger axonal diameter, that are correlated with conduction speed^{13,14}. In light of this previous work, we suggest that the left deviation in the line bisection we observed in most subjects may result from unbalanced speed of visuospatial processing between the two hemispheres along the SLF II. To test this hypothesis, we asked subjects to complete a modified Posner paradigm¹⁵ in which they detected as quickly as possible targets that appeared either in their left or right hemifield. In most subjects, the correlation analysis indicated that larger SLF II volumes in the right hemisphere corresponded to faster detection times in the left hemifield ($r = -0.471$; $P = 0.042$) (**Fig. 2c**). Correlations with SLF I ($r = 0.271$; $P = 0.262$) or SLF III ($r = -0.271$; $P = 0.262$) were not statistically significant. Moreover, larger deviation toward the left in the line bisection corresponded to faster detection times in the left hemifield ($r = 0.495$; $P = 0.031$) (**Fig. 2d**).

The correlations between the lateralization of the SLF II, the deviation observed in the line bisection and the detection times represent the first demonstration, to our knowledge, that anatomical asymmetry of the parieto-frontal connections predicts behavioral performance on visuospatial attention tasks. These findings may help to interpret the neuroanatomical basis of models of visuospatial attention based on functional imaging and studies of individuals who show clinical neglect. The cortical projections of the SLF I overlap with the dorsal

network activated during the voluntary orienting of spatial attention toward visual targets, and the SLF III overlaps with the ventral network that is activated during the automatic capture of spatial attention by visual targets⁸ and damaged in people with visuospatial neglect (**Supplementary Fig. 8**). In contrast, the SLF II overlaps with the parietal component of the ventral network and the prefrontal component of the dorsal network. Thus, our findings suggest that the SLF II represents a direct communication between the dorsal and ventral networks. This communication may act as a modulator for the dorsal network, redirecting goal-directed attention mediated by the SLF I to events identified as salient by the SLF III⁸.

In conclusion, we report the existence of a bilateral parieto-frontal network, previously described only in monkey, whose hemispheric lateralization predicts the degree of specialization of the right hemisphere for visuospatial attention. Our results also suggest that this hemispheric specialization is associated with an unbalanced speed of visuospatial processing along the SLF II. This lateralization may be predictive of visuospatial recovery in patients with lesions of parieto-frontal networks.

Note: Supplementary information is available on the Nature Neuroscience website.

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AUTHOR CONTRIBUTIONS

M.T.d.S. conceived and coordinated the study, reviewed and collected neuropsychological data, performed the tractography dissections, helped with the post-mortem dissections and wrote the manuscript. F.D. developed the spherical deconvolution algorithm, collected and preprocessed the neuroimaging data before the dissections and helped drafting the manuscript. S.J.F. helped collecting neuropsychological data and drafting the manuscript. A.S. and D.G.M.M. provided funding for the neuroimaging data and helped to draft the manuscript. F.V. helped drafting the manuscript and performed the post-mortem dissections. M.C. helped to conceive and coordinate the study. M.C. also wrote the manuscript and performed the post-mortem dissections.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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A Lateralized Brain Network for Visuospatial Attention

Supplementary Methods and Results

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A – SUPPLEMENTARY METHODS

1- Spherical Deconvolution (SD) tractography.

New developments in diffusion imaging tractography¹ offer a unique opportunity to visualize the organization of human brain pathways and to verify the existence of anatomical connections previously described only in the monkey²⁻⁴. Diffusion imaging is a modification of conventional magnetic resonance imaging sequences that permits the quantification of the diffusion characteristics of water molecules inside biological tissues¹. Given that cerebral white matter contains axons, and that water molecules diffuse more freely along axons than across them⁵, it is possible to obtain *in vivo* estimates of white matter fiber orientation by measuring the diffusivity of water molecules along different directions⁶. Tractography algorithms are used to reconstruct white matter tracts in three dimensions by sequentially piecing together discrete and shortly spaced estimates of fiber orientation to form continuous trajectories^{2, 4}. Tractography has previously been used to dissect a large number of white matter connections in the human brain and these *in vivo* reconstructions are very close to the post-mortem findings derived from classical blunt dissections^{7, 8}. However, the existence of a dorsal parieto-frontal system network similar to that described in the monkey^{9, 10} has proven difficult to ascertain in the human brain¹¹. This is due to the limitations of current tractography methods based on the diffusion tensor model, which provide only a single average estimate of the fiber orientation for each voxel. In voxels with crossing, kissing or fanning fibers, the tensor model is therefore unable to describe the complexity of the white matter organization and the resultant tractography reconstructions are likely to contain artefacts^{4, 12}. Spherical Deconvolution (SD) tractography partially overcomes these limitations by modelling the diffusion signal as a distribution of multiple fiber orientations. Therefore with SD the number and orientations of different fiber populations can be identified and quantified within each voxel¹³. For further information on the SD method see¹³.

2- Subjects

Twenty healthy right-handed volunteers (11 males and 9 females) aged between 22-38 years were recruited. All subjects gave written consent and were left-to-right readers. Handedness was estimated using the Edinburgh handedness test¹⁴, which ranges from -100 for extremely left handed to +100 for extremely right-handed participant. A semi-structured interview was used to exclude those subjects with a previous history of neurological and psychiatric disorders. None of the participants were on medication. Demographic data and test results are reported in Supplementary Table 1.

Supplementary Table 1: Demographic data, performances and confidence intervals (CI 95%) of the participants

Participant	Age	Sex	Handedness	Bisection(mm)± CI 95%	Reaction time(index)± CI 95%	SLF I (index)	SLF II (index)	SLF III (index)
S01	37	M	+80	2.4 ± 1.68	0.0165 ± 0.0407	-0.0162	-0.0481	0.3968
S02	30	M	+100	-3.1 ± 1.61	-0.0353 ± 0.0411	0.3135	0.0908	0.3469
S03	27	M	+100	-4.0 ± 1.01	0.0075 ± 0.0358	0.1677	0.0406	0.5750
S04	23	F	+60	0.4 ± 1.10	0.0430 ± 0.0297	0.2238	-0.1235	-0.0136
S05	32	M	+100	-1.0 ± 4.58	-0.0418 ± 0.0729	0.0737	0.0606	0.2597
S06	27	F	+100	-6.7 ± 1.94	-0.0638 ± 0.2042	-0.2032	0.1696	0.3998
S07	32	F	+100	0.2 ± 1.77	0.0129 ± 0.0733	-0.0778	-0.1604	0.2733
S08	28	F	+100	-0.4 ± 1.49	0.0167 ± 0.0893	-0.0241	0.2322	0.0903
S09	25	F	+100	-3.7 ± 1.01	-0.0327 ± 0.0402	-0.3189	0.2480	0.1784
S10	28	F	+100	-1.1 ± 1.12	-0.0246 ± 0.0459	-0.1421	0.1336	0.2328
S11	35	M	+100	-3.0 ± 2.28	-0.0110 ± 0.0377	-0.1127	0.2420	0.5540
S12	25	F	+100	-0.2 ± 1.17	0.0249 ± 0.0371	-0.2952	-0.0472	0.3699
S13	22	M	+100	1.6 ± 1.15	0.0302 ± 0.0241	0.2253	-0.2553	-0.1421
S14	26	M	+100	-1.2 ± 0.70	0.0158 ± 0.0680	0.0236	0.0516	0.1333
S15	32	M	+100	-1.7 ± 1.13	-0.0305 ± 0.1769	0.2169	0.0825	0.3101
S16	38	M	+100	-2.6 ± 2.09	-0.0562 ± 0.0823	-0.3732	0.1461	0.1568
S17	24	F	+100	-1.1 ± 1.07	-0.0081 ± 0.0923	-0.0029	-0.0824	0.5794
S18	25	M	+100	-2.7 ± 1.34	0.0157 ± 0.0701	-0.1670	0.1455	0.0346
S19	25	M	+100	0.8 ± 1.54	0.0216 ± 0.1956	0.3377	-0.3207	0.2552
S20	33	F	+100	-3.5 ± 0.98	0.0216 ± 0.0867	-0.0074	0.2477	0.2287

3- MRI data acquisition

For each participant, 60 contiguous near-axial slices were acquired on a 3T GE Signa HDx TwinSpeed system (General Electric, Milwaukee, WI, USA) with the following parameters: rostro-caudal phase encoding, voxel size 2.4x2.4x2.4 mm, matrix 128x128, slices 60, NEX 1, TE 93.4 ms, b-value 3000 s/mm², 60 diffusion-weighted directions and 7 non-diffusion-weighted volumes, using a spin-echo EPI sequence. Peripheral cardiac gating was applied with effective TR of 20/30 R-R intervals.

A sagittal three-dimensional MPRAGE data set covering the whole head was also acquired (166 slices, voxel resolution=1.2x1x1 mm, TE=2.8 ms, TR=7 ms, flip angle= 8°).

4- Diffusion MRI data processing

4.1- Correction for motion and eddy current distortion and estimation of the fiber orientation

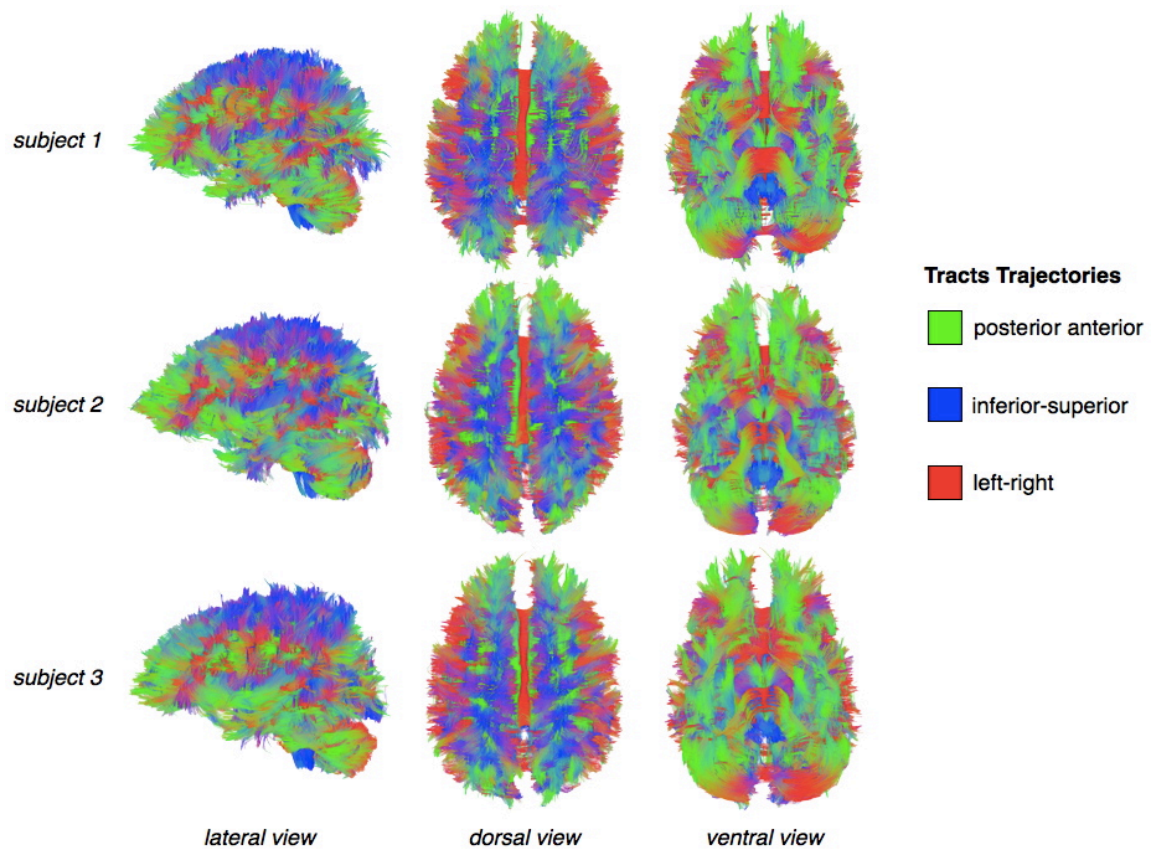
Diffusion datasets were corrected for head motion and eddy current distortions using affine registration to a non diffusion-weighted reference volume¹⁵ as implemented in the FSL software package¹⁶.

White matter orientation estimation was performed using a spherical deconvolution (SD) approach^{17, 18}. SD was chosen to estimate multiple orientations in voxels containing different populations of crossing fibres¹⁹. SD was applied using a modified (damped) version of the Richardson-Lucy algorithm¹³. The damped Richardson-Lucy algorithm reduces partial volume effects and spurious fiber orientations by providing reliable estimates of the fiber orientation distribution (FOD) in voxels, which include mixed contributions of white matter, grey matter and cerebro-spinal fluid. Algorithm parameters were chosen as described before¹³. Fiber orientation estimates were obtained selecting the orientation corresponding to the peaks (local maxima) of the FOD profiles. To exclude spurious local maxima we applied an absolute and a relative threshold. A first "absolute" threshold was used to exclude small local maxima due to noise or isotropic tissue. This

threshold is three times the amplitude of a spherical FOD obtained from a grey matter isotropic voxel. A second "relative" threshold of 5% of the maximum amplitude of the FOD was applied to remove the remaining local maxima with values greater than the absolute threshold²⁰.

4.2- Tractography algorithm

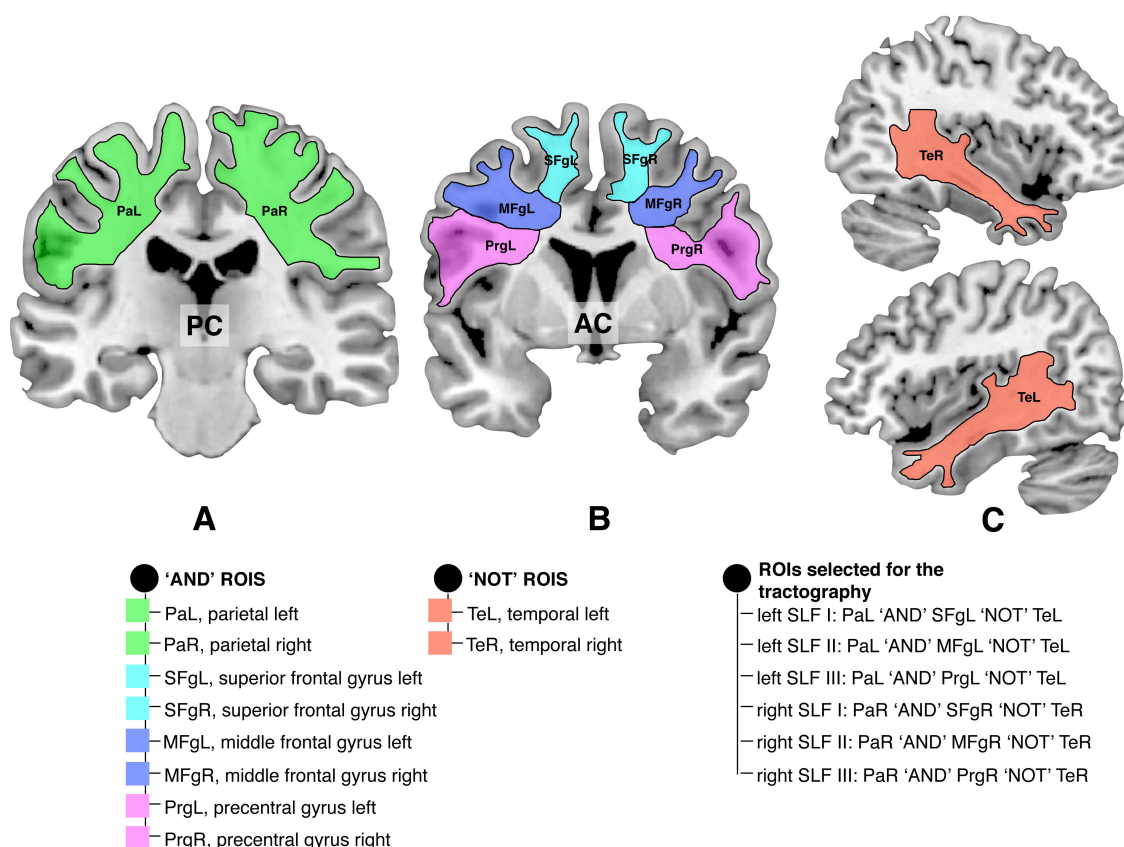
Whole brain tractography was performed selecting every brain voxel with at least one fiber orientation as a seed voxel. From these voxels and for each fiber orientation a modified fiber assignment by continuous tracking (FACT) algorithm³ was used to reconstruct streamlines²¹. Streamlines were reconstructed by sequentially piecing together discrete and shortly spaced estimates of fiber orientation to form continuous trajectories. When entering a region with crossing white matter bundles the algorithm followed the orientation vector of least curvature²². Streamlines were halted when a voxel without fiber orientation was reached or when the curvature between two steps exceeded a threshold of 45°. The software estimating and reconstructing the orientation vectors and the trajectories from diffusion MRI was written in Matlab 7.8 (<http://www.matwork.com>). Full brain tractography of three representative subjects are displayed using TrackVis²³ (<http://www.trackvis.org>) as shown in Supplementary Figure 1.



Supplementary Figure 1: Whole brain SD tractography using modified FACT algorithm of three representative subjects.

5- Parieto-frontal connections. Tractography dissections

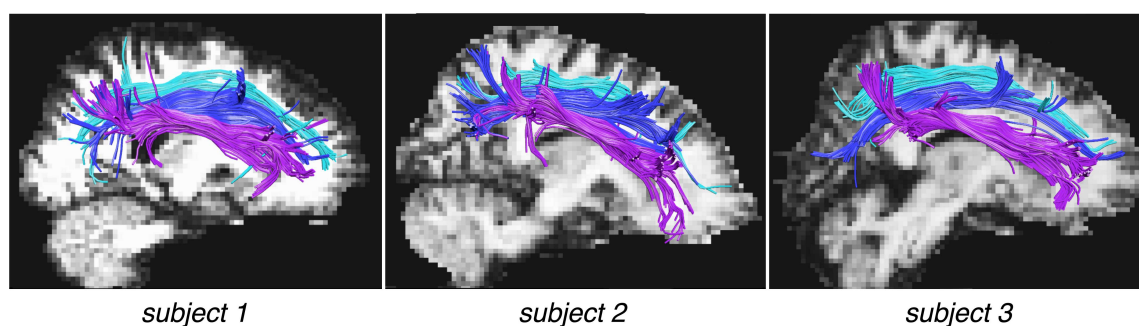
5.1- Segmentation of the subcomponents of the parietal-frontal pathways



Supplementary Figure 2: Delineation of the regions of interest (ROI) used for the tractography of the three subcomponents of the left and right parieto-frontal connections. (A) Parietal ROIs in the left (PaL) and right (PaR) hemispheres. (B) Frontal ROIs in the left (SFgL, MFgL and PrgL) and right (SFgR, MFgR and PrgR) hemispheres. (C) Temporal ROI used to exclude the connections belonging to the temporo-frontal arcuate fasciculus in the left (TeL) and the right (TeR) hemispheres.

A multiple region of interests (ROIs) approach was used to isolate different components of the parieto-frontal network. Three ROIs were delineated around the white matter of the superior, middle and inferior/precentral frontal gyri, and another three 'AND' ROIs were delineated posteriorly in the parietal region. Streamlines of the arcuate

fasciculus projecting to the temporal lobe were excluded using a ‘NOT’ ROI in the temporal white matter (the arcuate is not part of the parieto-frontal system as it projects to the temporal lobe) (Supplementary Figure 2). Results from the three representative subjects are shown in Supplementary Figure 3. There was no significant difference between the number of voxels in the left and right superior frontal gyri ROIs (SFG; $t_{(19)} = -1.306$; $p = 0.207$), middle frontal gyri ROIs (MFG; $t_{(19)} = -0.036$; $p = 0.972$), precentral gyri ROIs (Prg; $t_{(19)} = -0.821$; $p = 0.422$), parietal ROIs (Pa; $t_{(19)} = -0.343$; $p = 0.735$) and temporal ROIs (Te; $t_{(19)} = 1.274$; $p = 0.218$).

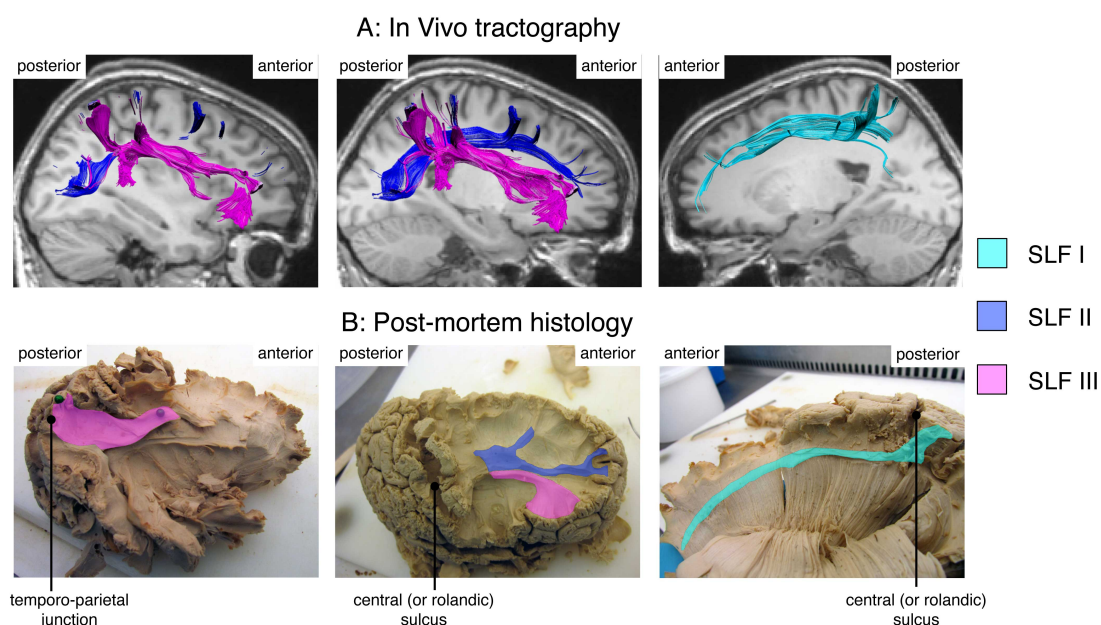


Supplementary Figure 3: Tractography reconstruction of the three branches of the parieto-frontal connections in three subjects. The most dorsal component (shown in light blue), the middle component (shown in navy blue) and the ventral component (shown in purple) correspond to the first, second and third branch of the superior longitudinal fasciculus (SLF I, II and III) respectively described in the monkey brain^{9, 10}.

5.2- Post-mortem validation of the SLF I, II and III.

Human post-mortem Klingler dissections²⁴ of the three SLF branches were performed on the right hemisphere, obtained from the autopsy of a 80 year-old woman's brain. This hemisphere was fixed in formalin for at least one year and then frozen at -15°C for two weeks. As described in Martino et al.²⁵ the water crystallization induced by the frozen process disrupts the structure of the gray matter and spreads the white matter fibers, facilitating the dissection of the fiber tracts. The SLF III and SLF II were exposed using

lateral surface to medial surface dissections. The SLF I was exposed using medial surface to lateral surface dissections (Supplementary figure 4).



Supplementary Figure 4: Side-by-side comparison of the SLF I (in light blue), II (in navy blue) and III (in purple) obtained after a virtual dissection of a human living brain (A) and a real dissection of a post-mortem brain (B).

6- Atlasing the three branches of the SLF in the stereotaxic space.

6.1- Mapping of the pathway.

For each component of the parietal-frontal network binary visitation maps were created by assigning each voxel a value of 1 or 0 depending on whether the voxel was intersected by the streamlines of the tract²⁶⁻²⁸. These maps were normalized to the Montreal Neurological Institute (MNI) stereotaxic atlas and smoothed (full width half maximum=4*4*4) using SPM (<http://www.fil.ion.ucl.ac.uk/spm>). The smoothed and normalized visitation maps were then entered into a design matrix for a one-sample t-test corrected for Family Wise Error (FWE). Full details of this approach are given in²⁹. Coronal sections of the result are shown in main Figure 2.

6.2- Cortical projections

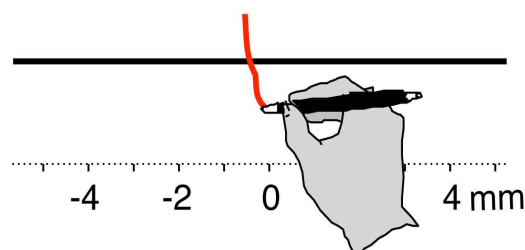
To visualize the cortical projections of the three components of the parieto-frontal networks Trackvis software was used to extract the endpoint of each streamline. Binary visitation maps of the tractography endpoints for each subcomponent of the SLF were normalized to MNI space and smoothed using the same approach described above. The smoothed and normalized tractography endpoints maps were then entered into a design matrix for a one-sample t-test corrected for Family Wise Error (FWE). Results were projected onto the average 3D rendering of the internal cortical layer of the 20 participants in figure 1C. A similar approach has been reported in³⁰. Supplementary Table 2 reports the T values and the coordinates of the local maxima of each cluster revealed by the group effect analysis.

Supplementary table 2: T value and MNI coordinates at the local maxima of each significant cluster of the SLF I, II and III

Parieto-frontal Subcomponent	Cluster volume (mm3)	Coordinates (mm,)			T value	p value(cluster) FWE corrected
		x	y	z		
SLF I Left	15538	-9	-45	53	8.34	0.001
	12724	-15	26	32	5.54	0.001
SLF I Right	14324	5	-43	52	5.15	0.001
	11826	17	22	42	5.27	0.001
SLF II Left	2980	-27	-44	30	5.15	0.014
	2916	-31	6	48	5.54	0.015
SLF II Right	7591	38	-59	23	5.11	0.001
	6181	32	14	40	4.47	0.001
SLF III Left	6314	-45	-42	31	7.52	0.001
	4426	-44	10	13	4.83	0.001
SLF III Right	10167	47	-36	35	18.80	0.001
	20671	41	13	15	7.72	0.001

7- Behavioral measures

7.1- Line bisection



Supplementary Figure 5: Diagram of the line bisection

The line bisection paradigm consisted of twenty cm long, 1-mm thick black lines centered on a horizontal A4 sheet (one line per sheet) presented aligned to the subjects' eye-axis, in a central position relative to the patient's sagittal head plane. Subjects were instructed to mark the center of each line with a pencil. Each subject marked 10 lines in total, 5 with the left hand and 5 with the right hand. The deviation from the true centre was recorded and an average of the performance with both hands was used to perform correlation analysis with the tractography lateralization indexes.

7.2- Posner paradigm



Supplementary Figure 6: Diagram of the modified Posner paradigm

The reaction time task consisted of a modification of the Posner paradigm³¹. The display contained a central fixation point and two boxes (unfilled squares) one on each side of the screen. The participants were asked to fixate on the central point and were instructed to press a button with the right hand each time they saw a star appearing in one of the two boxes. The reaction time (RT) was recorded in milliseconds. Before the appearance of the star, an arrow was briefly presented that pointed either to the left or right. The whole session consisted of 50% of trials with a valid cue (arrow pointing in the direction of the

star) and 50% of trials with an invalid cue (arrow pointing in the opposite direction of the star). The inter-trial interval was randomized between 4760-9440 ms.

8- Statistical analysis.

Statistical Analysis. Statistical analysis was performed using SPSS software (SPSS, Chicago, IL). A lateralization index was calculated for the volume of the three branches of the SLF according to the following formula:

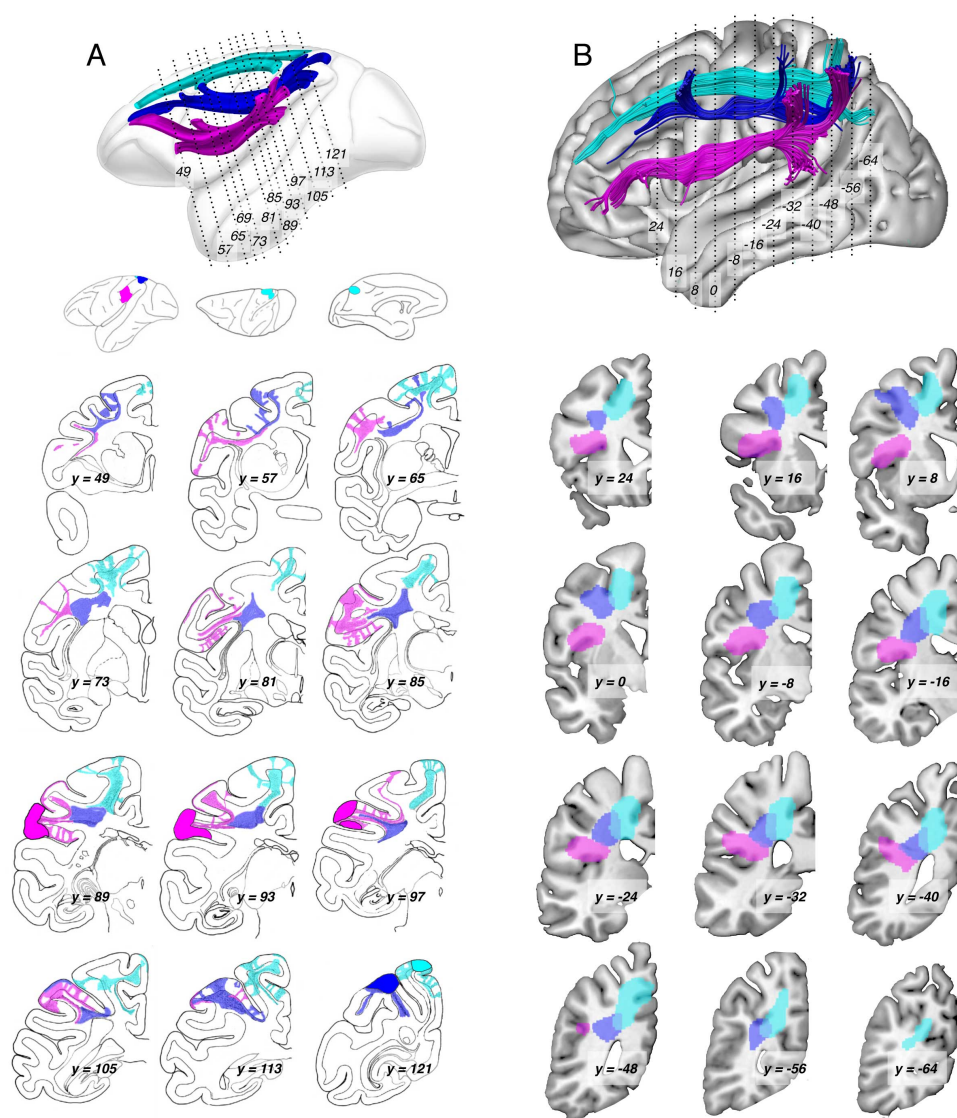
$$\text{Lateralisation index} = (\text{Right volume} - \text{Left volume}) / (\text{Right volume} + \text{Left volume}).$$

Negative values indicate a leftward volume asymmetry and positive values a rightward asymmetry. A lateralization index of the detection time was calculated in a similar manner to that calculated for the volume of the SLF.

In our analysis, Gaussian distribution was confirmed for all the dependant variables using the Shapiro–Wilk test. This allows the use of standard parametric statistics in our dataset to draw statistical inferences. A one-sample t test (test value = 0) was used to assess the lateralization of the volume in voxels of the SLF I, II and III. Pearson correlation analysis was performed between the lateralization index of the three branches of the superior longitudinal fasciculus (volume in voxels) and the behavioral performances. We identified 1 outlier for the line bisection (more than two standard deviation from the mean) that we removed from the correlation analyses.

B – SUPPLEMENTARY RESULTS

1- Rhesus Monkey SLF I, II and III

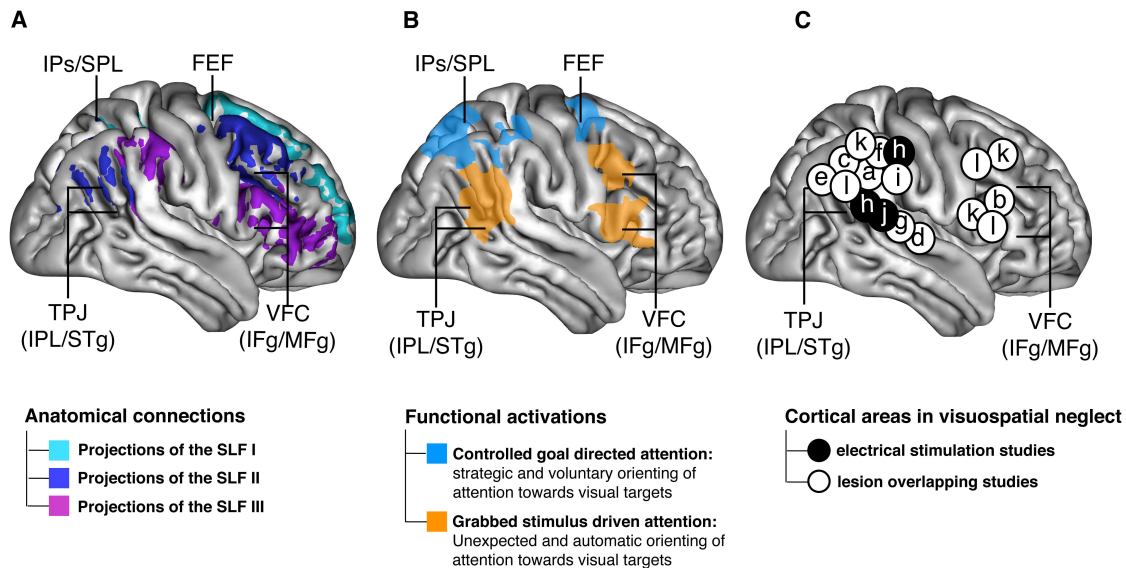


Supplementary Figure 7: Reconstruction of the three branches of the SLF: comparison between post-mortem axonal tracing in monkey (A)^{9, 10} and in vivo SD tractography in humans (B).

The monkey maps of the SLF I, II and III presented in supplementary Figure 7 are modified from the coronal slices provided in an Atlas by Schmahmann & Pandya¹⁰. The modification consists of coloring the tract in light blue (SLF I), navy blue (SLF II) and purple (SLF III) according to the site of injection for the axonal tracing. Projection and commissural fibers have been removed for the purpose of visualization.

Supplementary Figure 7 shows side by side the virtual in vivo dissections from our study and corresponding slices from a monkey atlas¹⁰ that we have modified for direct comparison. Overall parieto-frontal connections of the human and the monkey brain are organized similarly in three longitudinal pathways. In humans, the most dorsal pathway originates from the precuneus and the superior parietal lobule (Brodmann areas, BA 5 and 7); and projects to the superior frontal and anterior cingulate gyri (BA 8, 9 and 32). This pathway corresponds to the first branch of the superior longitudinal fasciculus (SLF I) as described in the monkey brain^{9, 10, 22}. In contrast the middle pathway originates in the anterior intermediate parietal sulcus and the angular gyrus (BA 39 and 40) and ends in the posterior regions of the superior and middle frontal gyri (BA 8 and 9). This pathway corresponds to the SLF II in the monkey brain^{9, 10, 22}. Lastly, the most ventral pathway originates in the temporo-parietal junction (BA 40) and terminates in the inferior frontal gyrus (BA 44, 45 and 47); corresponding to the SLF III in the monkey brain^{9, 10, 22}. We were also able to replicate our in vivo findings using post-mortem blunt dissections in a human brain (see supplementary Figure 4). Although the post-mortem dissections were limited in identifying the exact cortical projections of the three SLF, a good correspondence was found between post-mortem and in vivo dissections of the central course of the three branches. Overall our results suggest a strong similarity between monkey and human parieto-frontal connections.

2- Functional activation studies



Supplementary Figure 8: The parieto-frontal networks for visuo-spatial attention as identified by tractography (A), functional neuroimaging (B) and brain lesion/electrical stimulation (C) studies. The SLF I projects to areas activated in tasks requiring controlled goal directed attention, whereas the SLF III projects towards areas activated during tasks requiring automatic reorienting of spatial attention to unexpected stimuli. The cortical projections of the SLF II overlap with both dorsal and ventral functional networks (B adapted from³²). Disorders of spatial attention are frequently associated with either cortical or subcortical lesions of the ventral parieto-frontal network (C) a³³, b³⁴, c³⁵, d³⁶, e³⁷, f³⁸, g³⁹, h⁴⁰, i⁴¹, j⁴², k⁴³, l⁴⁴ IPs: intraparietal sulcus; SPL: superior parietal lobule, FEF: frontal eye field, TPJ: temporo-parietal junction, IPL: inferior parietal lobule, STg: superior temporal gyrus, VFC: ventral frontal cortex, IFg: inferior frontal gyrus, MFg: middle frontal gyrus.

The figure summarizing the functional activation studies (fMRI and PET) presented in supplementary figure 8 was adapted from the work of Corbetta et al.³². In particular the studies involved tasks for the detection of cortical functional activation during two conditions: i) strategic and voluntary orienting of spatial attention towards visual targets⁴⁵⁻⁴⁸; ii) unexpected and automatic orienting of attention towards visual targets⁴⁹⁻⁵⁴. Foci of activation reported in Corbetta et al.³², were projected onto the average 3D rendering of the internal cortical layer of the 20 participants used in our study.

3- Brain lesion studies

The summary figure of the brain lesion and electrical stimulation studies presented in Figure 2 was created using all the studies previously published in the literature. A comprehensive search of group studies with PUBMED identified 10 brain lesion studies a³³, b³⁴, c³⁵, d³⁶, e³⁷, f³⁸, g³⁹, i⁴¹, k⁴³, l⁴⁴ and 2 intraoperative electrical stimulation studies h⁴⁰ and j⁴². Foci of maximum overlap of the lesion or electrical stimulation were projected onto the average 3D rendering of the internal cortical layer of the 20 participants.

C – SUPPLEMENTARY NOTE

Although the SLF III shows the most significant rightward lateralization, this did not correlate with the line bisection performance and the speed of visuospatial processing. A possible explanation may be that the SLF III has a key role in sustained attention and novelty processing, which have not been measured in our study^{32, 55, 56}. Furthermore the function of the SLF III differs between the two hemispheres. In the left hemisphere the SLF III projects to areas involved in verbal fluency⁵⁷ and praxis⁵⁸. In the right hemisphere the SLF III projects to areas involved in visuospatial attention³², prosody⁵⁹ and music processing⁶⁰. Hence, this suggests that an anatomical asymmetry of the brain should not be taken as direct evidence of hemispheric dominance as the correlation between anatomical lateralization and specialization of functions is not straightforward.

Further, in this study the number of voxels visited by the reconstructed streamlines was used as a surrogate measure of the tract volume. It is, however, important to highlight that the number of visited voxels does not represent the true tract volume but rather quantifies the space occupied by the reconstructed tract. Hence, factors such as multiple fiber crossing, fanning or kissing⁴ can affect the ability to reconstruct streamlines and therefore the overall volume estimate. In this study we used SD tractography to minimize this bias by tracking through regions with multiple fiber orientations. It is however still possible that smaller branches of the SLF were not reconstructed due to the relatively large voxels size resolution of our datasets.

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Special issue: Research report

Short frontal lobe connections of the human brain

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ABSTRACT

Advances in our understanding of sensory-motor integration suggest a unique role of the frontal lobe circuits in cognition and behaviour. Long-range afferent connections convey higher order sensory information to the frontal cortex, which in turn responds to internal and external stimuli with flexible and adaptive behaviour. Long-range connections from and to frontal lobes have been described in detail in monkeys but little is known about short intralobar frontal connections mediating local connectivity in humans. Here we used spherical deconvolution diffusion tractography and post-mortem dissections to visualize the short frontal lobe connections of the human brain. We identified three intralobar tracts connecting: i) posterior Broca's region with supplementary motor area (SMA) and pre-supplementary motor area (pre-SMA) (i.e., the frontal 'aslant' tract – FAT); ii) posterior orbitofrontal cortex with anterior polar region (i.e., fronto-orbitopolar tract – FOP); iii) posterior pre-central cortex with anterior prefrontal cortex (i.e., the frontal superior longitudinal – FSL fasciculus system). In addition more complex systems of short U-shaped fibres were identified in the regions of the central, pre-central, perinsular and fronto-marginal sulcus (FMS). The connections between Broca and medial frontal areas (i.e. FAT) and those between the hand-knob motor region and post-central gyrus (PoCG) were found left lateralized in a group of twelve healthy right-handed subjects. The existence of these short frontal connections was confirmed using post-mortem blunt dissections. The functional role of these tracts in motor learning, verbal fluency, prospective behaviour, episodic and working memory is discussed. Our study provides a general model for the local connectivity of the frontal lobes that could be used as an anatomical framework for studies on lateralization and future clinical research in neurological and psychiatric disorders.

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1. Introduction

In the last two centuries the role attributed to the frontal lobes has progressively expanded from pure motor execution (Fritsch and Hitzig, 1870; Ferrier, 1875) to more complex functions such as attention and memory (Stuss et al., 1999; Fuster, 2009; Reilly et al., 2011), executive cognition (Fuster, 2009; Krause et al., 2012; Zappala' et al., 2012; Cubillo et al., 2012; Tsermentseli et al., 2012), social behaviour (Shamay-Tsoory et al., 2010; Sundram et al., 2012; Langen et al., 2012) and consciousness (Crick and Koch, 1990; Dehaene et al., 1998). This wide range of abilities relies on multiple networks of fibres composing the intricate anatomy of the frontal white matter (Yeterian et al., 2012; Thiebaut de Schotten et al., 2012). Through long-range projection and association fibres the frontal lobes receive sensory information from subcortical nuclei (e.g., thalamus) and sensory cortices (i.e., visual, auditory, somatosensory, gustatory and olfactory) and respond to environmental stimuli. These connections are also used to exert top-down control over sensory areas (Fuster, 2009). Shorter fibres that mediate the local connectivity of frontal lobes include U-shaped connections between adjacent gyri and longer intralobar fibres connecting distant areas within the same lobe (Yeterian et al., 2012). The anatomy and the functional correlates of these short frontal fibres are largely unknown in man. Therefore, our study aims at using tractography and post-mortem dissections to visualise these short connections of the human frontal lobes.

The short connections of the human brain were described in some detail by Theodor Meynert in the second half of the 19th Century. He attributed to the short U-shaped connections a central role in human cognition and correctly identified them as cortico-cortical short association connections of different lengths:

'The cortex exhibits on the convexity of each convolution the shape of an inverted U, which is changed in the next adjoining fissure to an upright U (top and bottom of the cortical wave)... The depressed surface of a cortical wave can be easily dissected out as from a smooth medullary groove, which on closer inspection is seen to consist of U-shaped medullary fibres... The U-shaped bundles of the cortex do not necessarily extend simply from one convolution to the one next adjoining, but they may skip one, two, three, or an entire series of convolutions... The shortest *fibrae propriae* lie nearest to the cortex.' (Meynert, 1885).

Meynert did not specify a pattern of distribution of these fibres and his anatomical observations led him to conclude that such U-shaped connections are ubiquitous in the brain. A decade later Heinrich Sachs produced a detailed atlas of the U-shaped fibres of the occipital lobe where he was able to identify and name prominent short connections organised in larger bundles visible on post-mortem dissections. Among these the U-shaped connections between the upper and lower banks of the calcarine sulcus (i.e., stratum calcarinum) and the dorsal to the ventrolateral occipital cortex (i.e., stratum profundum convexitatis) (Sachs, 1892). Unfortunately, Sachs limited his anatomical investigations to the occipital lobe leaving the mapping of the U-shaped connections of the entire human brain incomplete.

At the turn of the 19th Century, experimental studies in animals (Fritsch and Hitzig, 1870; Ferrier, 1875; Broca, 1861; Bianchi, 1895) and clinical observation in patients with aphasia (Broca, 1861) and epilepsy (Jackson, 1915) attracted the interest of anatomists to the frontal lobe (Catani and Stuss, 2012). In 1906 Cristfield Jakob described a system of longitudinal U-shaped fibres connecting adjacent frontal gyri (Jakob, 1906). He also described a 'brachial center' and a 'facio-lingual center' in the pre-central gyrus (PrCG) connected to parietal post-central cortex through direct U-shaped connections. It is unfortunate that Jakob's work on the frontal U-shaped fibres was published in Spanish and had scarce diffusion in the English literature (Theodoridou and Triarhou, 2012).

An original approach to short fibre mapping was made by Rosett who produced an atlas of short connections of the human brain (Rosett, 1933). His method consisted in the immersion of a previously fixed brain in a gas-compressed tank containing liquid carbon dioxide (CO₂). After quickly opening the valve of the tank the sudden reduction of pressure transforms the liquid CO₂ into a gas. The micro-explosions of the cerebral tissues cause a mechanical separation of the fibres along natural lines of cleavage. With this method Rosett described the main orientation of the short fibres of most the gyri and sulci of the human brain, but he was not able to visualize their entire course and terminal projections.

In more recent years the study of U-shaped connections continued in animals by means of axonal tracing studies. Yeterian et al. (2012) give a comprehensive account of the short frontal lobe connections in monkey. However, the significant differences between species in the anatomy and function of the frontal lobes suggest that probably translating *tout court* findings from axonal tracing to humans can be not as straightforward as previously thought (Thiebaut de Schotten et al., 2012).

Preliminary diffusion imaging tractography studies have reported U-shaped connections of the frontal lobes in the living human brain (Conturo et al., 1999; Oishi et al., 2008; Lawes et al., 2008; Guevara et al., 2011; Catani et al., 2002). These studies represent an important advancement in our understanding of human connective anatomy but they need validation.

The present study aims at mapping the architecture of short frontal lobe tracts in the human brain by combining post-mortem blunt dissections (Klingler, 1935) and diffusion tractography based on spherical deconvolution (Dell'acqua et al., 2010; Thiebaut de Schotten et al., 2011a). This combined approach and in particular the use of spherical deconvolution models offers advantages that partially overcome the limitations of classical tractography (Catani, 2007; Thiebaut de Schotten et al., 2011b). The visualization of the tracts as Digital Dejerine maps (see methods section) facilitates the anatomical description of the short U-tracts.

2. Methods

2.1. MRI acquisition and preprocessing

Diffusion weighted MR data was acquired using a High Angular Resolution Diffusion Imaging (HARDI) acquisition optimized for

spherical deconvolution (Dell'Acqua et al., 2010; Tournier et al., 2004). A total of 70 near-axial slices were acquired from a 29-year-old, right-handed healthy subject on a Siemens Trio 3.0 T equipped with a 32-channel head coil. The acquisition sequence was fully optimized for advanced diffusion-weighted imaging, providing isotropic ($2 \times 2 \times 2$ mm) resolution and coverage of the whole head. At each slice location, three images were acquired with no diffusion gradient applied, together with 64 diffusion-weighted images in which gradient directions were uniformly distributed in space. The acquisition was peripherally-gated to the cardiac cycle with an echo time (TE) = 85 msec and repetition time (TR) equivalent to 24 RR intervals. The diffusion weighting was equal to a b -value of 2000 s/mm^2 . To increase the signal to noise ratio (SNR) the whole acquisition was repeated four times. Raw diffusion-weighted data were up-sampled to $1 \times 1 \times 1$ mm with a 3rd order b -spline interpolation. The four datasets were concatenated and simultaneously registered and corrected for subject motion and geometrical distortions using ExploreDTI (<http://www.exploredti.com>) (Leemans and Jones, 2009). An axial three-dimensional MPAGE dataset covering the whole head was also acquired (176 slices, $1 \times 1 \times 1$ mm isotropic resolution, TE = 4.2 msec, TR = 2.3 msec, flip angle = 9°).

In addition to the single dataset, diffusion MRI data were also acquired from 12 right handed, healthy and normal volunteers using a 3T GE Signa HDx TwinSpeed system (General Electric, Milwaukee, WI, USA). Diffusion weighted spin-echo single shot EPI images were acquired with the following parameters: voxel size $2.4 \times 2.4 \times 2.4$ mm, matrix 128×128 , FOV = 307×307 mm, 60 slices, 1 NEX, TE 93.4 msec, b -value 3000 s/mm^2 , 60 diffusion-weighted directions and seven non-diffusion-weighted volumes, using a spin-echo EPI sequence with an ASSET factor of 2. Peripheral gating was applied with an effective TR of 20/30 R–R intervals. These datasets were acquired to analyze lateralization of tracts related to manual dexterity and language.

2.2. Data processing

The diffusion data was then processed using a spherical deconvolution approach based on the damped version of the Richardson Lucy algorithm as described in (Dell'acqua et al., 2010). The high SNR of the data allowed us to apply a relatively low regularisation threshold equal to $\eta = .02$ without an excessive increase of spurious components in the fibre orientation distributions (FODs). The other parameters for the deconvolution algorithm were: i) a fibre response function equivalent to a tensor of $[1.5 \ 0.3 \ 0.3] \times 10^{-3} \text{ mm}^2/\text{s}$; 200 algorithm iterations and ii) a regularisation geometric parameter of $\nu = 8$. Fibre orientation estimates were obtained by selecting the orientation corresponding to the peaks (local maxima) of each FOD profile. To exclude spurious local maxima, we applied an absolute and a relative threshold. A first “absolute” threshold was used to exclude small local maxima due to noise or isotropic tissue. This threshold is three times the amplitude of a spherical FOD obtained from a grey matter isotropic voxel. A second “relative” threshold of 5% of the maximum amplitude of the FOD was applied to remove the remaining local maxima with values greater than the absolute threshold (Dell'Acqua et al., 2009).

Whole brain tractography was performed selecting every brain voxel with at least one fibre orientation as a seed voxel.

From these voxels and for each fibre orientation streamlines were propagated using an Euler integration with a step size of .5 mm and an angular threshold of 45° . When entering a region with crossing white matter bundles, the algorithm follows the orientation vector of least curvature as described in Schmahmann et al. (2007). Streamlines were halted when a voxel without fibre orientation was reached or when the curvature between two steps exceeded a threshold of 45° .

Digital Dejerine Maps were obtained by constraining tractography in non-contiguous brain slices of 2 mm (Axial, Sagittal, Coronal). Tractography was started from 10 seed points randomly placed inside each brain voxel and for each fibre orientation. Streamlines were propagated as in the whole brain tractography following fibre orientations using Euler integration with a step size of .5 mm and an angular threshold of 45° . Tractography propagation was arbitrary stopped after 40 mm. This enhances visualization of the white matter bundles that propagate along the plane of the slice selected. Bundles that are oriented perpendicularly to the surface of the slice are visualized only as dots or very short streamlines. Tractography maps were finally visualized using a lookup table empirically tuned to simulate historical black-and-white anatomical drawings. All data processing was performed using in-house software developed with MATLAB (The MathWorks, Inc., Natick, MA). Visualization was performed using TrackVis (www.trackvis.org).

2.3. Tractography dissections

Virtual dissections were performed in TrackVis using two-ROIs to isolate single tracts (Catani and Thiebaut de Schotten, 2008). Virtual dissections were systematically performed for each sulcus and frontal gyrus following a posterior to anterior order (e.g., central sulcus, pre-central sulcus, etc.). Spheres were used to isolate single tracts as shown in Fig. 1. All tracts presented were dissected on both hemispheres. A lateralization index of the volume of the fronto-parietal U-shaped tracts and premotor connections was calculated using the following formula: $(\text{Right Volume} - \text{Left Volume}) / (\text{Right Volume} + \text{Left Volume})$. Negative values indicate a left lateralization. Two-tails, unpaired samples, t -test was used to assess statistical significance of lateralization indices.

2.4. Post-mortem dissections

Post-mortem dissection of white matter fibres was performed according to the technique originally described by Klingler (1935). One right hemisphere was obtained from the autopsy of an 80-year-old healthy woman. The specimen was fixed in 10% formalin solution for at least three months. After removal of the pia-arachnoid membrane and cortical vessels, the hemisphere was frozen at -15°C for 15 days. The water crystallization induced by the freezing process disrupts the structure of the grey matter (which has a high water content), thus making it easier to peel off the cortex from the brain surface. The freezing process also separates the white matter fibres, thus facilitating their dissection. The specimen was washed under running water for several hours before performing the dissection (Martino et al., 2010).

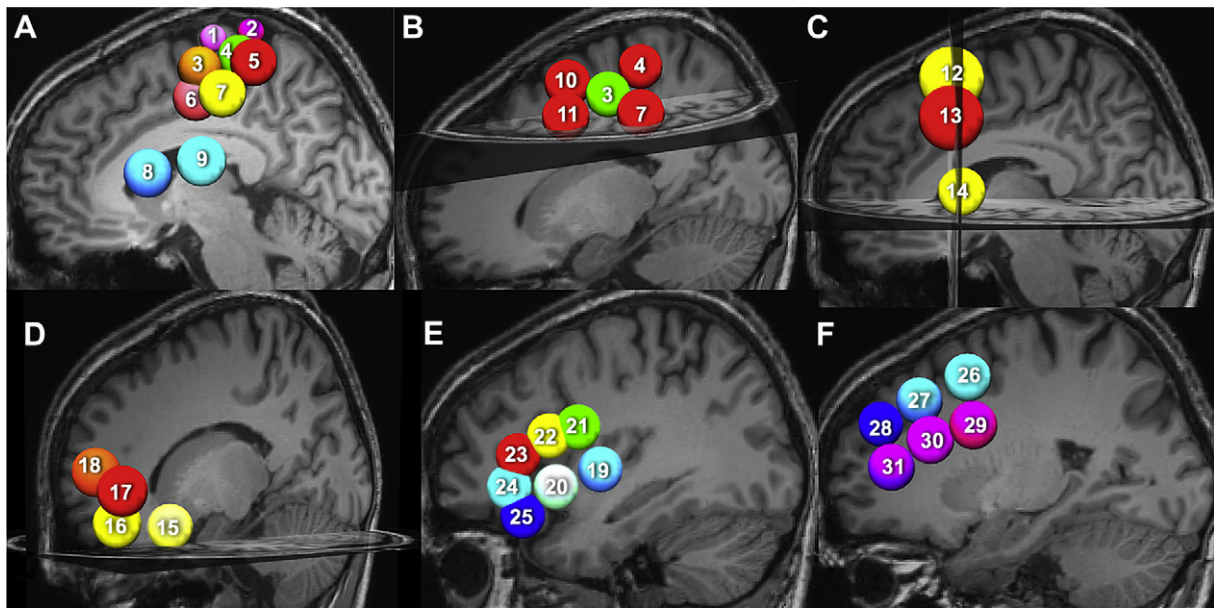


Fig. 1 – Placement of the regions of interest (spheres) used to dissect the frontal tracts. Note that the spheres' colours correspond to those of the tracts shown in Figs. 3–8. A) Regions of interest for the paracentral U-tracts (1 and 2), hand superior (3 and 4), middle (3 and 7) and inferior (5 and 6) U-tracts, and face/mouth U-tracts (8–9). B) Regions of interest for the U-tracts connecting the hand-knob motor region with the postcentral gyrus (3 and 4 for the hand superior and 3 and 7 for the hand middle) and superior (3 and 10) and middle frontal gyri (3 and 11). C) Regions of interest for the frontal aslant tract (FAT) (12 and 14) and the U-shaped tracts connecting the superior and middle frontal gyri (12 and 13) and the inferior and middle frontal gyri (13 and 14). D) Regions of interest for the fronto-orbitopolar (FOP) (15 and 16) and fronto-marginal tracts (FMT) (17 and 18). E) Regions of interest for the fronto-insular tracts (FIT) connecting the posterior insula with the subcentral gyrus (19 and 21) and the anterior insula with the pre-central (20 and 22), pars opercularis (20 and 23), pars triangularis (20 and 24) and pars orbitalis (20 and 25) of the IFG. F) Regions of interest for frontal longitudinal system (FLS) composed of the frontal superior longitudinal (FSL) (26, 27 and 28) and frontal inferior longitudinal (FIL) (29, 30 and 31) tracts.

The superficial anatomy of the brain was studied in detail, with identification of the sulci and gyri. The dissection was then started, with removal of the cortex and exposure of the underlying U-fibres (also known as intergyral or arcuate fibres). The white matter dissection was then completed in a stepwise manner, from lateral to medial. Wooden spatulas were used in the initial step of the dissection, to peel away the brain cortex. Once the U-fibres were identified, the dissection was performed using blunt metallic dissectors with different tip sizes. Care was taken to separate the fibres using the blunt edge of the instrument, thus avoiding the generation of spurious tracts. Metal pins were used to indicate anatomical landmarks and digital pictures were taken during the dissection.

3. Results

The 3D reconstruction of the frontal lobe surface and corresponding cytoarchitectonic areas according to Brodmann's division (Brodmann, 1909) are shown in Fig. 2. The surface landmarks (i.e., sulci and gyri) and cytoarchitectonic areas (Ono et al., 1990; Catani et al., in press) are used to describe the anatomy of the dissected tracts and their projections. Tractography reconstructions of the short frontal lobe tracts are presented in Figs. 3–9, whereas Digital Dejerine maps and post-mortem dissections are shown in Figs. 10–12. A diagram

that summarises the local connectivity and some of the long-range connections of the frontal lobe is presented in Fig. 13.

3.1. Fronto-parietal U-tracts (FPUTs) (central sulcus connections)

A chain of U-shaped connections between the frontal PrCG and the parietal PoCG was identified (Fig. 3). These tracts can be divided into three groups:

- i) The paracentral lobule tract has its convexity oriented medially towards the interhemispheric midline and connects the frontal and parietal portions of the paracentral lobule. This tract connects pre- and post-central regions corresponding to the 'foot' area of the motor-sensory homunculus (purple tract in Fig. 3).
- ii) The hand-knob region contains connections composed of three separate tracts with different orientation; a superior tight U-shaped tract with an anterior-posterior course (green tract in Fig. 3), a ventral tract with an oblique anterior-posterior course (red tract in Fig. 3) and a transverse middle U-shaped tract with a dorsal to ventral course and an upwards concavity (yellow tract in Fig. 3). These three tracts connect pre- and post-central regions corresponding to the hand area of the motor-sensory homunculus.

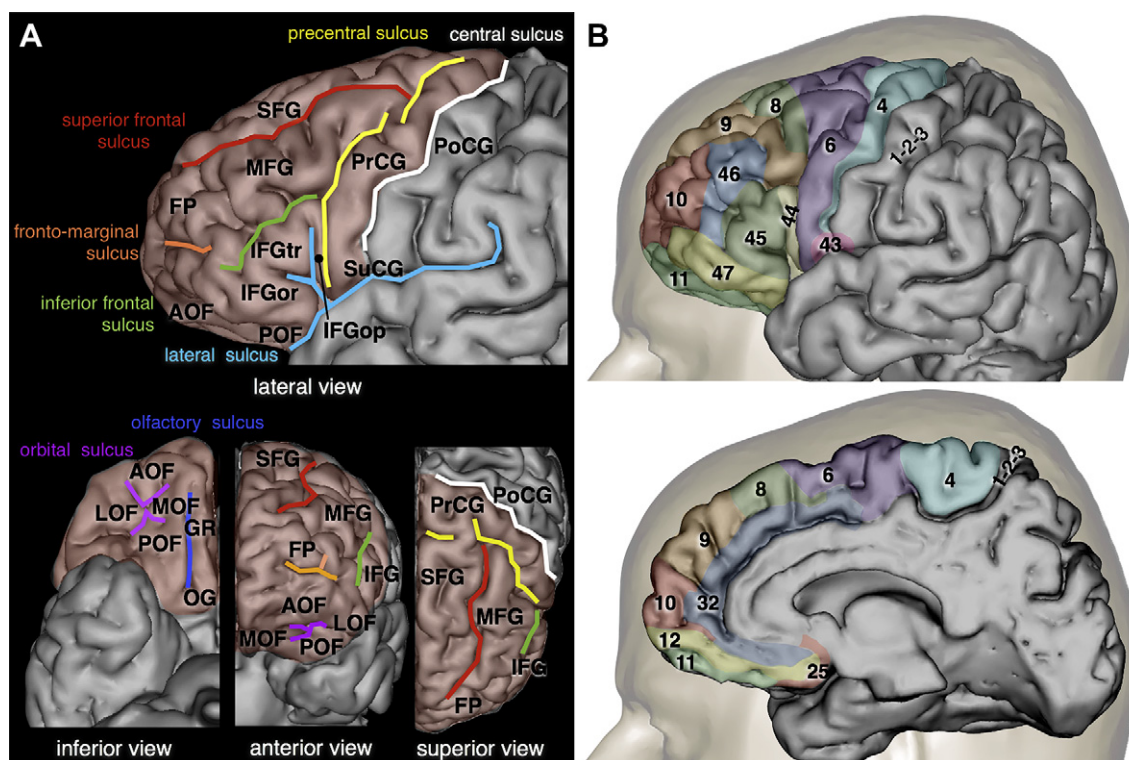


Fig. 2 – A) Surface anatomy and B) cytoarchitectonic areas (according to Brodmann, 1909) of the frontal lobe. AOF, anterior orbitofrontal gyrus; GR, gyrus rectus; IFGop, inferior frontal gyrus pars opercularis; IFGor, inferior frontal gyrus pars orbitalis; IFGtr, inferior frontal gyrus pars triangularis; LOF, lateral orbitofrontal gyrus; MOF, medial orbitofrontal gyrus; OG, olfactory gyrus; POF, posterior orbitofrontal gyrus; SFG, superior frontal gyrus; SuCG, subcentral gyrus.

iii) The ventral group of connections is composed of two or more tracts characterised by a more oblique and elongated course compared to the other dorsal tracts of the foot and hand region. These tracts connect pre- and post-central regions corresponding to the face, mouth and tongue area of the motor-sensory homunculus (light and dark blue tracts in Fig. 3).

To investigate the overall local connectivity of the hand-knob region dissections of the U-shaped tracts between pre-central and premotor regions were performed (Fig. 4). A group of four U-shaped tracts connects the hand-knob region to post-central sensory cortex and premotor cortex of the superior and middle frontal gyri. These tracts are circularly arranged around the long projection fibres (green tracts in Fig. 4) of the hand region like the ‘petals’ of a poppy flower. The concavity of the posterior and inferior ‘petals’ corresponds to the bed of the central sulcus in the hand region. These two tracts correspond to the superior and middle fronto-parietal connections of the hand-knob region described in the previous section (i.e., hand superior and middle U-tracts). Another two U-tracts connect the PrCG to more anterior premotor cortex. Of these two anterior tracts the superior ‘petal’ that connects PrCG to the superior frontal gyrus (SFG) passes beneath the junction between the posterior branch of the superior frontal sulcus and the pre-central sulcus (frontal eyefield region). The anterior ‘petal’ connects the PrCG to the middle frontal gyrus (MFG). The long

connections of the hand-knob region are surrounded by the short U-tracts and are composed of ascending thalamic projections and descending cortico-striatal (putamen), cortico-pontine and cortico-spinal tracts.

3.2. Frontal aslant tract (FAT) and premotor connections

The posterior region of the superior and inferior frontal gyri is interconnected by a direct system of fibres forming the ‘frontal aslant tract’ (yellow tract in Fig. 5). This tract projects to the anterior supplementary and pre-supplementary motor area (pre-SMA) of the SFG and the pars opercularis of the inferior frontal gyrus (IFG). Some projections reach also the pars triangularis of the IFG and the inferior region of the PrCG. These two regions are also interconnected through U-shaped fibres running superficially to the FAT and projecting to the posterior portion of the MFG (red tracts in Fig. 5). Finally these three cortical regions of the superior, middle and inferior frontal gyri are directly connected to the striatum (caudate and putamen) through a system of radial projection fibres (blue tracts in Fig. 5).

3.3. Fronto-orbitopolar (FOP) and fronto-marginal connections

Two prominent intralobar tracts project to the frontal pole (FP) (Fig. 6). The FOP runs on the ventral aspect of the frontal lobe and connects the posterior orbital gyrus to the anterior orbital

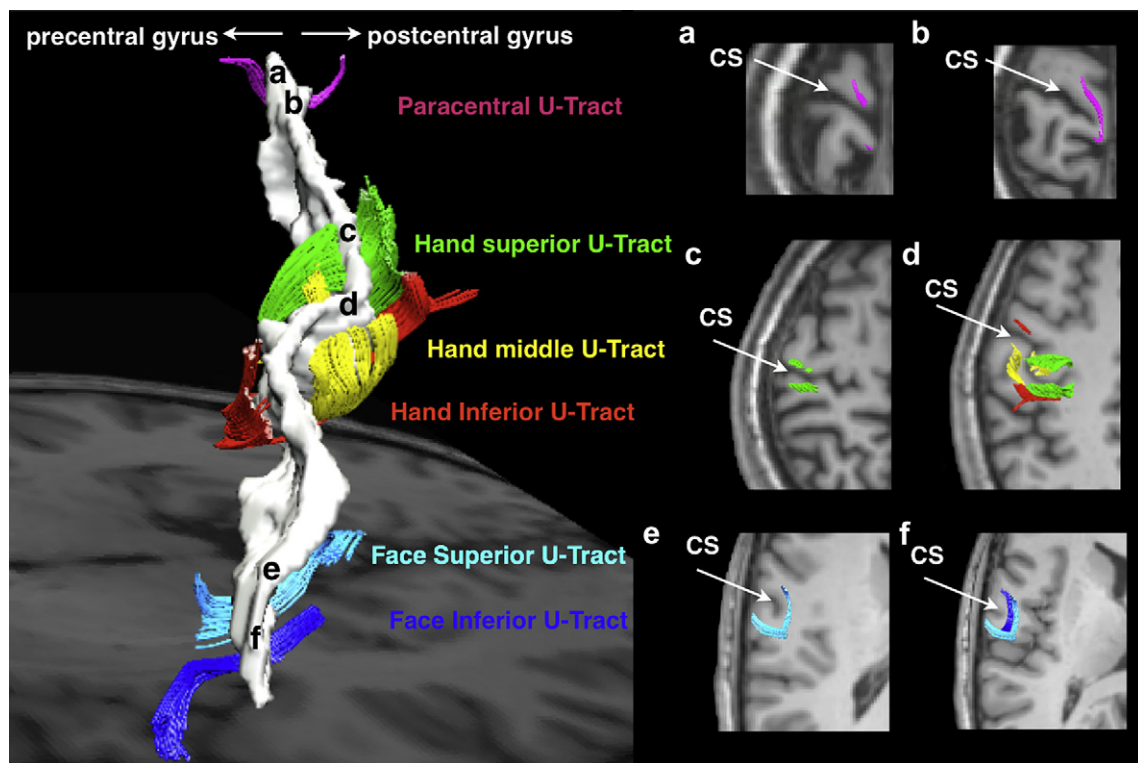


Fig. 3 – Left, lateral view of the short fronto-parietal U-tracts (FPUT) connecting pre-central and post-central gyri. The white region corresponds to the central sulcus (CS). Letters a–f indicate the level of the axial slices on the right panel.

gyrus and ventromedial region of the FP (yellow tract in Fig. 6). The fronto-marginal tract (FMT) runs beneath the fronto-marginal sulcus (FMS) and connects medial and lateral regions of the frontopolar cortex (red tract in Fig. 6). The cortical regions connected by these short intralobar tracts receive afferent connections through the uncinate and inferior fronto-occipital fasciculi, anterior thalamic projections and send efferent connections to the striatum through the fronto-striatal tracts.

3.4. Fronto-insular tracts (FIT)

A system of U-shaped fibres organised around the peri-insular sulcus connects the inferior frontal and PrCG to the insula of Reil (Fig. 7). The most posterior tracts connect the subcentral lobule to the post-central long insular gyrus (green tract in Fig. 7). Anterior to this tract is a group of four U-tracts connecting the PrCG (yellow tract) and the pars opercularis (red tract), pars triangularis (light blue tract) and pars orbitalis (dark blue tract) of the IFG to the insular gyri anterior to the central sulcus of the insula. The fronto-insular fibres have their concavity always towards the insula.

3.5. Frontal longitudinal system (FLS)

A chain of U-shaped connections that resemble a prolongation of the superior longitudinal fasciculus connects the dorsolateral cortex of the premotor and prefrontal cortex (Fig. 8). Tracts of different length compose this parallel system. Some of these tracts are short and connect adjacent

gyri, others connect more distant regions. The majority of these tracts has a longitudinal course and are organised along a direction parallel to the superior and inferior frontal sulci. The superior chain (i.e., frontal superior longitudinal – FSL) connects the PrCG to the ventral part of the SFG and dorsal part of the MFG (light blue tracts in Fig. 8). The inferior chain (i.e., frontal inferior longitudinal – FIL) projects from the PrCG to the ventral part of the MFG and superior part of the IFG (purple tracts in Fig. 8). These two systems converge anteriorly to the same regions of the FP and along their course are interconnected by transversal U-shaped tracts.

3.6. Analysis of the volume asymmetry of the fronto-parietal and premotor connections

A significant leftwards asymmetry was found for the lateralization index of the fronto-parietal U-shaped tracts of the hand region ($t = -2.932$, $p = .014$) and the FAT ($t = -3.672$, $p = .004$) (Fig. 9). There were no statistically significant differences in the lateralization pattern of the dorsal ($t = -1.017$, $p = .331$) and ventral ($t = -.790$, $p = .446$) fronto-parietal U-shaped tracts and in the connections between superior and middle frontal gyri ($t = 1.331$, $p = .213$) and middle and inferior frontal gyri ($t = -1.620$, $p = .136$).

4. Discussion

Using a novel tractography approach based on spherical deconvolution and post-mortem blunt dissections, short

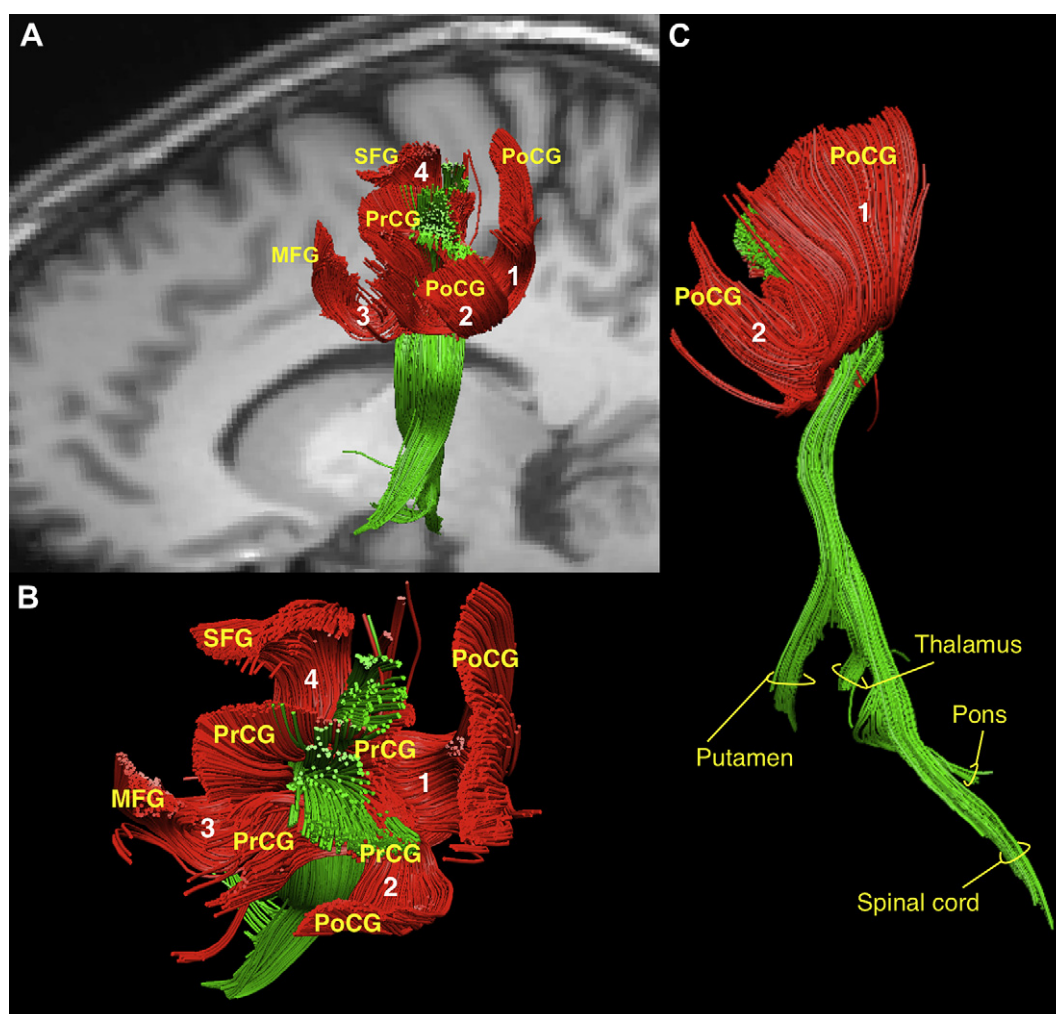


Fig. 4 – Reconstruction of the short U-shaped (red) and long projection (green) tracts of the hand-knob motor region in the left hemisphere. A) Left lateral view; B) top view C) posterior view. The connections of the hand region resemble a ‘poppy flower’ with a green stem and four red ‘petals’ (1, posterior; 2, inferior; 3, anterior; 4, superior). The posterior (1) and inferior (2) petals correspond to the fronto-parietal U-tracts (FPUT) between pre-central (PrCG) and post-central (PoCG) gyrus shown in Fig. 3 (i.e. hand superior and hand middle, respectively). The anterior (3) and superior (4) petals correspond to the U-shaped connections between the precentral gyrus (PrCG) and the middle frontal gyrus (MFG) and the MFG and superior frontal (SFG) gyrus, respectively. The ‘green stem’ is formed by ascending thalamo-cortical projection fibres and descending projections to the putamen (cortico-striatal), pons (cortico-pontine) and spinal cord (cortico-spinal tract).

frontal lobe connections of the human brain were identified. Spherical deconvolution has recently been developed to partially overcome the limitations of classical diffusion tensor imaging (Tournier et al., 2004; Dell’Acqua et al., 2007; Dell’acqua et al., 2010). This method has the ability to identify and quantify the orientation of different populations of fibres within a single voxel (Dell’acqua et al., 2010; Tournier et al., 2007). Hence, one of the advantages of tractography based on spherical deconvolution is the possibility of resolving fibre crossing and reducing false negative reconstructions of white matter pathways. This can facilitate the visualization of those connections that are not visible with diffusion tensor tractography (Thiebaut de Schotten et al., 2011a, b).

A preliminary indirect comparison with previous axonal tracing studies in the monkey brain (Schmahmann and Pandya, 2006) and post-mortem human investigations (Lawes

et al., 2008), including our own dissections, suggests that the majority of the short frontal fibres can be easily identified in the living human brain using tractography algorithms applied to acquisitions optimized for spherical deconvolution (Dell’acqua et al., 2010). Some of the large intralobar tracts and the short U-shaped fibres described in our study have also been reported using classical diffusion tensor tractography with both manual and automatic clustering methods (Lawes et al., 2008; Oishi et al., 2008; Guevara et al., 2011). A general feature of the U-shaped tracts is their distribution along the walls and floors of the major sulci of the frontal lobes. For this reason we have used a nomenclature based on the names of the sulci these fibres belong to (e.g., fronto-marginal tract). For other tracts interconnecting distant gyri or projecting to neighbouring lobes (i.e., insula, parietal) the regions of termination of the U-shaped tracts were used instead (e.g., fronto-insular tracts –

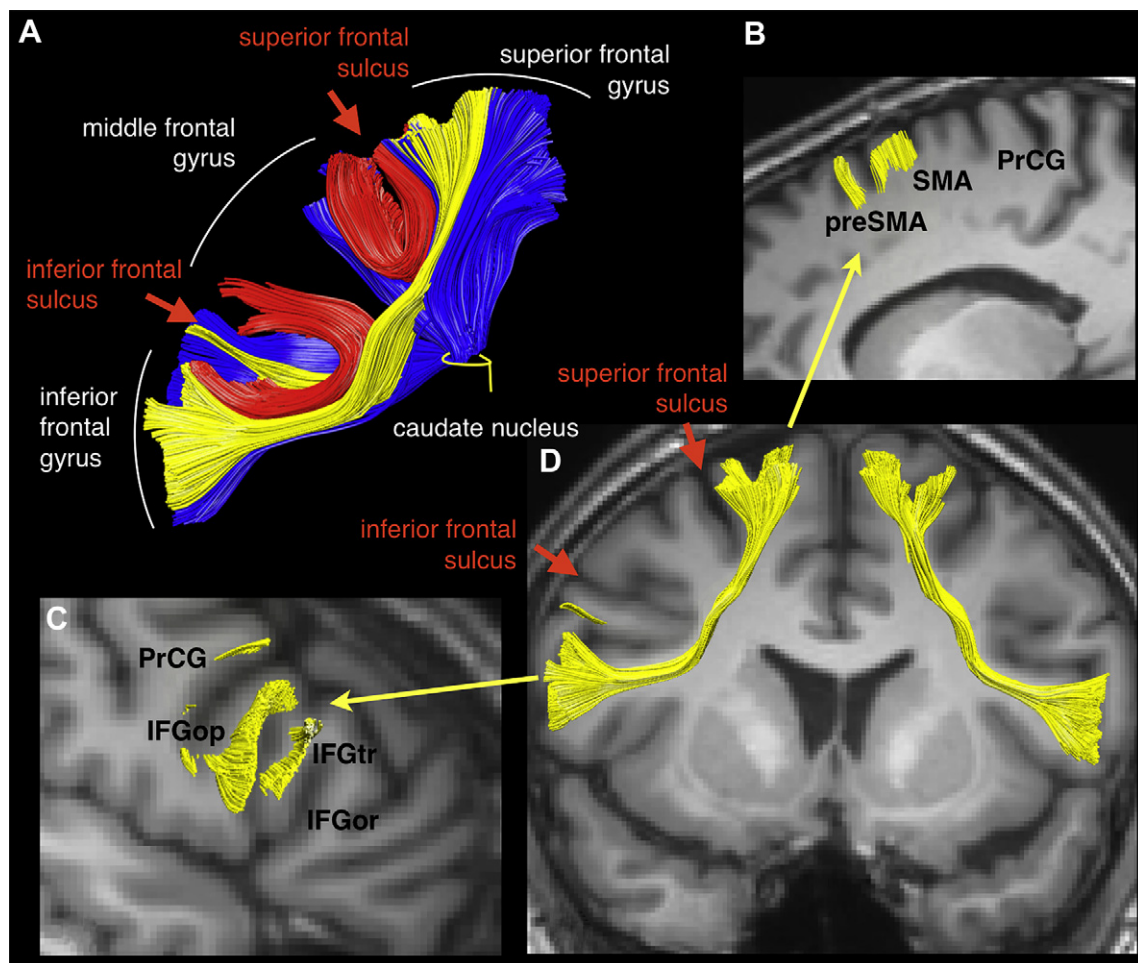


Fig. 5 – A) Connections of the premotor regions of the frontal lobe. The frontal aslant tract (FAT) (yellow) connects the B) dorsal and medial (supplementary and pre-supplementary motor area, SMA and pre-SMA) cortex of the SFG with the C) posterior region of IFG. Red U-shaped tracts connect the superior and middle frontal gyri and the inferior and middle frontal gyri. Blue projection fibres connect the cortical premotor regions with the head of the caudate nucleus. D) The frontal aslant is a bilateral tract (for the lateralization analysis see Fig. 9).

FITs). The only exception is the frontal ‘aslant’ tract that will be discussed below.

Overall the pattern of distribution of these tracts is not homogeneous but rather discontinuous along the course of each sulcus with some regions showing a higher number of interconnecting U-shaped tracts. This observation requires careful interpretation. Current diffusion methods are limited by the dimensions of the voxels and are therefore likely to underestimate the presence of smaller fibres. Hence, the ‘absence’ of direct U-shaped connections between adjacent gyri (e.g., pre-central and post-central connections are missing in a region between the dorsal paracentral lobule and the hand region as shown in Fig. 3) in the tractography reconstruction should not be interpreted as a complete anatomical absence of axonal fibres. Tractography provides only an indirect indication of the real spatial extension of the tract (i.e., ‘volume’) and the true anatomical connectivity between regions. Thus while we feel confident to conclude that, in relation to the number of streamlines or the space occupied by these trajectories, some regions are more likely to be interconnected compared to others, we avoid interpreting

the lack of streamlines as indicative of a complete absence of connections.

The exact functional role of the short U-shaped connections remains to be explained. Overall our study suggests that the distribution of the U-shaped fibres follows a functional division rather than a purely anatomical pattern. The three tracts of the central sulcus, for example, whose distribution and relative volume have a precise correspondence with the homunculus regions (Penfield, 1937), are probably in relation to the importance of sensory information for motor control of skilful movements of the hand, mouth/tongue and foot (Catani and Stuss, 2012). Our post-mortem dissections also suggest that the location of the U-shaped connections has a direct correspondence with some anatomical features of the surface anatomy of the gyri. The orientation and density of the U-shaped of the central sulcus, for example, have a precise correspondence with the presence of protuberances from the wall (i.e., ‘buttresses’) or the floor (i.e., ‘annectant convolutions’) of the central sulcus (Fig. 10) (Rosett, 1933). This correspondence could have practical implications for neuroradiologists, for example, or neurosurgeons intending to use surface landmarks

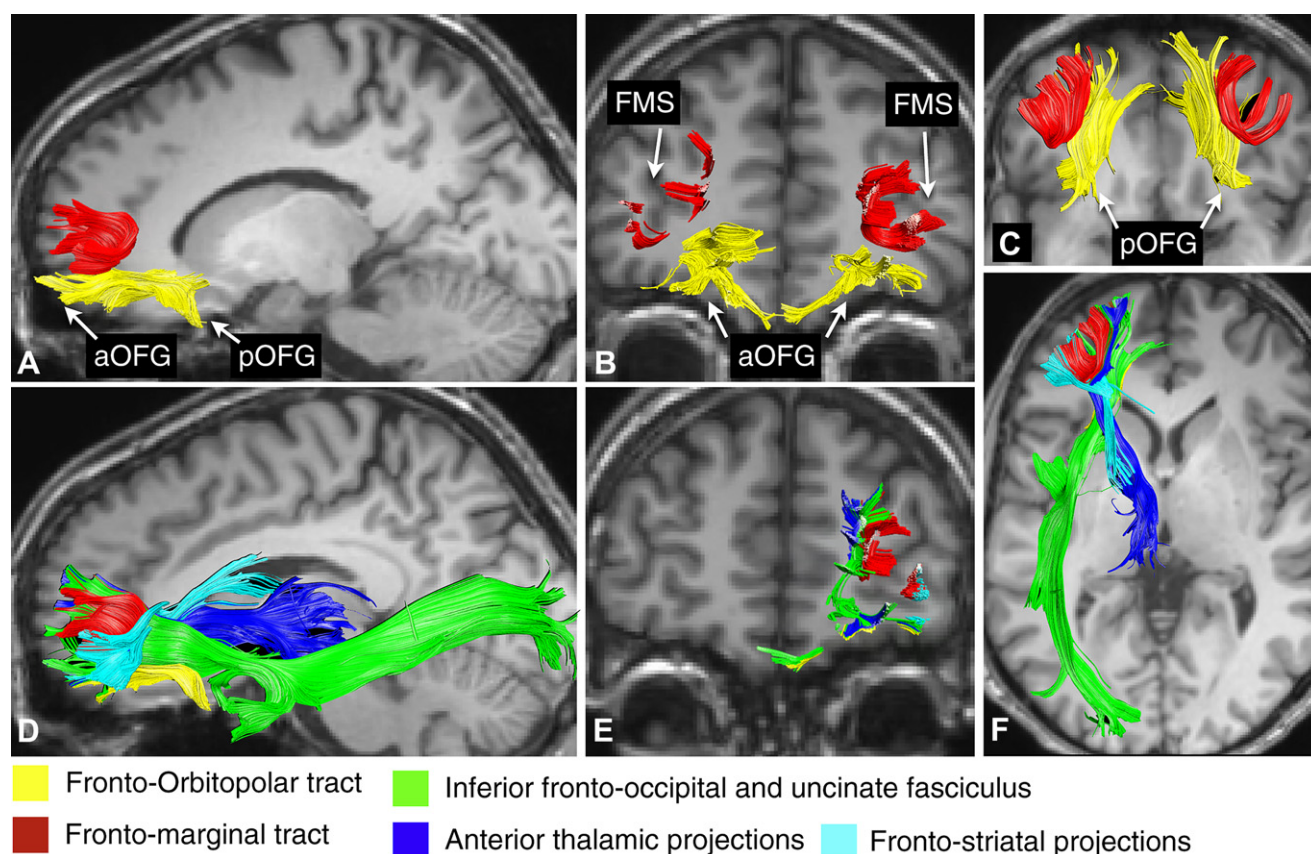


Fig. 6 – Intralobar and long frontal tracts of the frontal pole shown on sagittal (A and D) coronal (B and E) and axial (C and F) slices. The fronto-orbitopolar (FOP) tract (yellow) connects posterior (pOFG) and anterior (aOFG) orbitofrontal gyri and inferior polar cortex. The fronto-marginal tract (FMT) (red) connects medial and lateral regions of the frontal pole. The frontal pole communicates with posterior cerebral regions and subcortical nuclei through long association and projection fibres visualized in D–F together with the FOP and FMT.

to identify the underlying U-shaped fibres on conventional radiological images or during surgery.

4.1. FPUTs of the central sulcus

Direct connections between post-central sensory and pre-central motor cortex have been previously described in mice (Ferezou et al., 2007), cats (Sakamoto et al., 1987; Sakamoto et al., 1989) and monkeys (Pavlidis et al., 1993; Schmähmann and Pandya, 2006). Similar connections have been visualized in the human brain using post-mortem dissections (Rosett, 1933) and diffusion imaging tractography (Conturo et al., 1999; Shinoura et al., 2005; Guevara et al., 2011). It is surprising that direct connections between primary sensory and motor cortices are not considered to play a significant role in current models of sensory-motor integration, for example, in relation to grasping (Grafton, 2010; Davare et al., 2011).

The primary motor cortex receives direct modulatory somatosensory inputs from the thalamus during execution of movements. This input is relayed directly from the ventrolateral thalamic nucleus to the primary motor cortex through the ascending thalamic projections (Fig. 11) (Iriki et al., 1991). Hikosaka et al., 1985 have, however, demonstrated in the monkey that pharmacological inactivation of neurons in the

primary somatosensory area (S1) causes deficits in precision grasping. This suggests that the motor cortex can receive somatosensory thalamic inputs through an alternative pathway relaying in the primary somatosensory cortex. We suggest that the fronto-parietal U-shaped fibres represent the final connections of this alternative indirect somatosensory-motor pathway.

There is some evidence that the direct and indirect somatosensory-motor pathways may have different functional roles. Electrophysiological investigations of the effects of the inputs from S1 to the motor cortex suggest that direct local U-shaped connections from PoCG may play a role in motor learning by facilitating long-term potentiation (LTP) in motor neurons of the primary motor area (M1) (Sakamoto et al., 1987; Iriki et al., 1991). Sakamoto et al., 1987 applied titanic stimulation to areas of the sensory cortex in monkeys and recorded from the corresponding sites in the primary motor cortex, where they found an increased synaptic excitability for periods of up to 90 min after the stimulation. Iriki et al., 1991 were unable to induce LTP in the motor cortex by applying titanic stimulation to the ventrolateral nucleus of the thalamus, which projects directly to motor cortex. From both these experiments we can conclude that sensory connections relayed through S1 (an indirect thalamo-somatosensory-motor route) induce LTP in the motor cortex that helps to consolidate

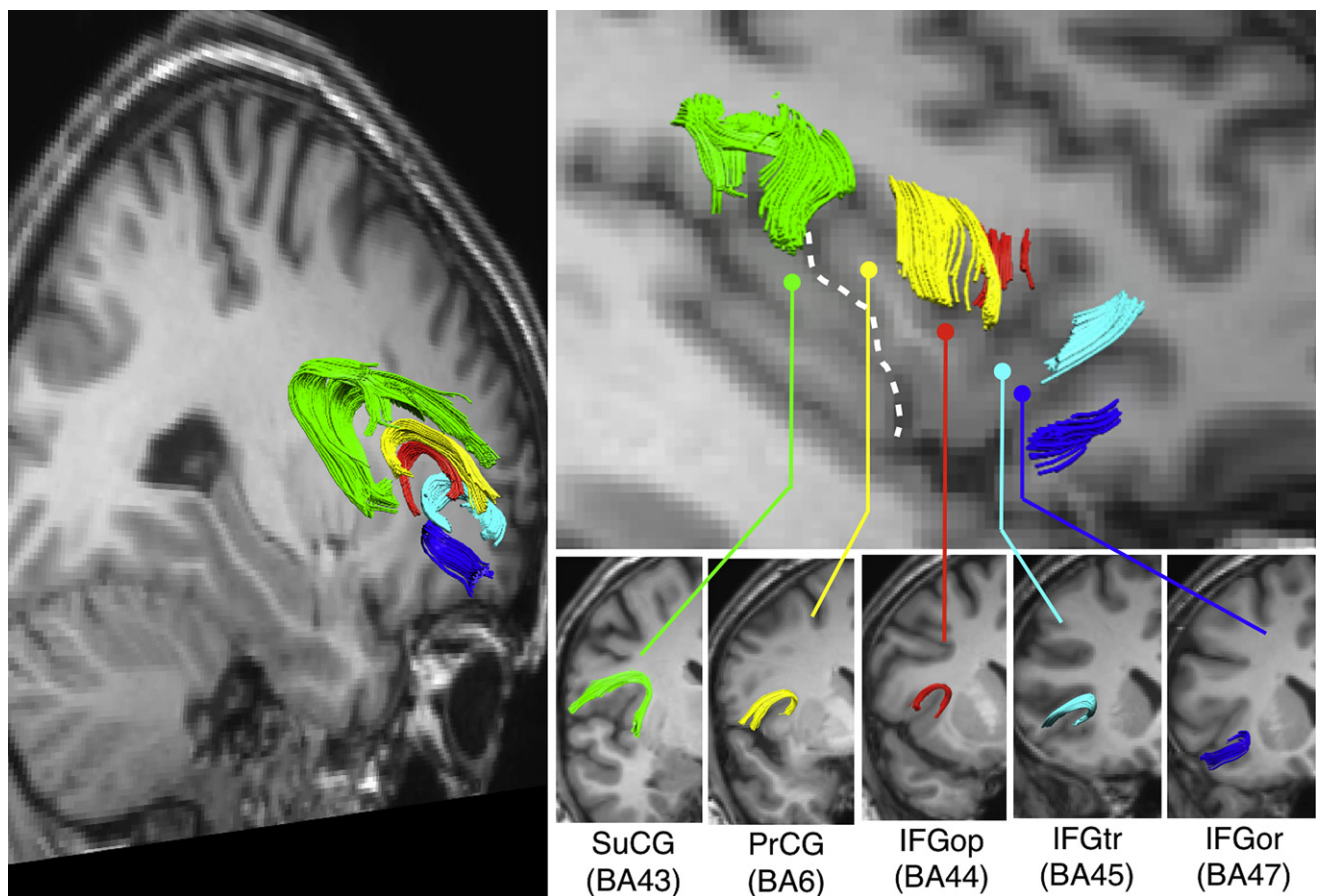


Fig. 7 – The fronto-insular system. This series of U-shaped tracts connect various regions of the frontal operculum with the insular cortex. The insula is divided into anterior and posterior part by the central sulcus of the insula which is indicated by the dash white line. All connections are with the anterior insula except for the connections from the sub-central gyrus (SuCG), which project to the posterior insula. IFGop – pars opercularis; IFGtr, inferior frontal gyrus – pars triangularis; IFGor, inferior frontal gyrus – pars orbitalis.

motor schemas and novel movement combinations. Pavlides et al., 1993 have provided further evidence for this hypothesis demonstrating that monkeys with ablated somatosensory cortex were unable to learn new motor skills, but were able to perform skills that had been learnt prior to surgery.

The pre-central and post-central U-shaped fibres are unlikely to convey other type of sensory information (e.g., visual) necessary for motor learning. Indeed, pharmacological inactivation of S1 neurons causes deficits in precision grasping but not in visually guided reaching or hand shaping (Hikosaka et al., 1985). The visual information is processed in the parietal regions posterior to S1 and is conveyed to more anterior frontal regions through the long association fibres of the superior longitudinal fasciculus system (Fig. 11) (Thiebaut de Schotten et al., 2011a, 2012). In the frontal lobe the visual information is used to elaborate complex body movements in the dorsal and ventral premotor regions located in the superior and middle frontal gyri. Our dissections show that the hand region in the PrCG receives direct U-shaped connections from these dorsal and ventral premotor areas. We speculate that the connections from the SFG are part of the ‘reaching’ network and the connections from the MFG belong to the ‘grasping’ circuit (Grafton, 2010; Davare et al., 2011). These U-shaped

connections are likely to carry visual information necessary to coordinate fine-tuning of finger movements with more complex reaching and grasping.

Our dissections also suggest a concentric organisation of the connections of the hand region, where the short association U-fibres are more peripherally distributed and surround the long projection fibres originating from the central core of the white matter of the PrCG. This is in keeping with the observation in the monkey brain of a central ‘cord’ composed of projection fibres surrounded by association fibres (Schmahmann and Pandya, 2006). Thus, it appears that the hand region is a central hub of the sensory-motor system where tactile and visual inputs converge for the online control of a complex cortico-cortical and cortico-subcortical network involving the fronto-parietal cortex, basal ganglia, thalamus, brain stem, cerebellum and spinal cord.

This system adapts dynamically to the task and re-organises with training and after injury. fMRI studies of musicians, for example, show that amateur players recruit a greater area of fronto-parietal cortex compared to professional players (Lotze et al., 2003; Karni et al., 1995). A combined fMRI and DTI study in a patient with a metastatic tumour in the primary motor cortex showed that recovery of motor

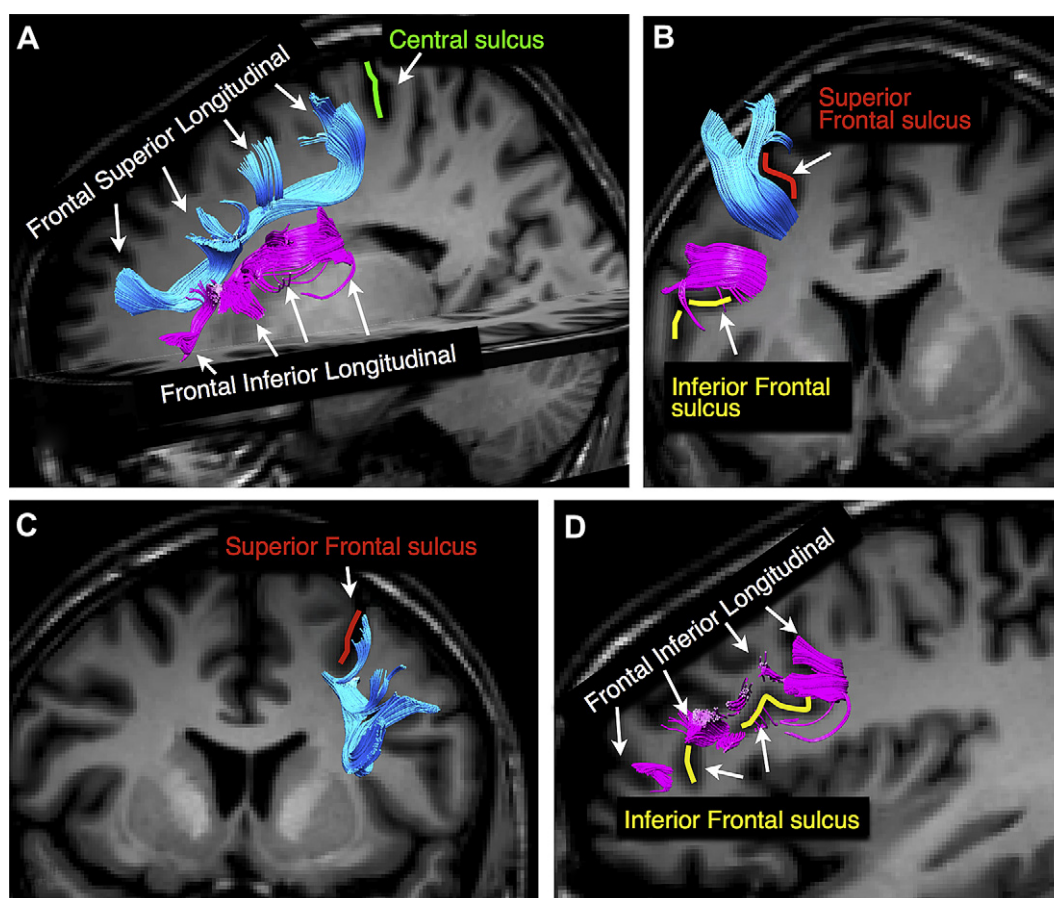


Fig. 8 – The frontal longitudinal system (FLS) is composed of a frontal superior longitudinal (FSL) (cyan) and a frontal inferior longitudinal (FIL) (purple) tract. These tracts are composed of short and long connections running along the superior and inferior frontal sulci and projecting mainly to the middle frontal gyrus. A) left lateral view; B) posterior view; C) anterior coronal view; D) left lateral view.

function after removal of the tumour and damage to the motor hand region was associated with increased activation of the post-central somatosensory area (Shinoura et al., 2005). Tractography after the operation showed interruption of the U-shaped fibres from the M1 suggesting a possible inhibitory effect of M1 on S1 through the U-shaped fibres of the central sulcus. Release of S1 from inhibitory control can lead to activation of somatosensory area and control of spinal motor neurons through the cortico-spinal fibres originating from extra-M1 cortex.

Most of the studies of sensory-motor integration have focused on hand and finger movements, but it is likely that the same conclusions apply to all U-shaped fibres between motor and somatosensory cortices. Our findings suggest, however, a greater volume of U-shaped connections for the hand region compared, for example, to surrounding motor cortex controlling proximal muscles (e.g., forearm). We interpret this finding as evidence of a higher local somatosensory to motor connectivity for those cortical regions controlling muscles involved in finely tuned movements and complex motor skills. This hypothesis is supported by experimental evidence of a greater influence of peripheral somatosensory inputs for movements of the distal muscles (e.g., hand) than proximal muscles (Lemon, 1981). This could also explain the observation of greater volume of the hand region compared to the mouth

and foot region (Fig. 9). The relatively smaller volume of the other two regions suggests the need of progressively minor amount of sensory-motor integration for the mouth and foot regions. Similarly one could argue that if the dominant hand commands a great sensory representation in the contralateral cortex, which allows for finer manipulation and in this case, the learning of more delicate manoeuvres, the lateralization of the U-fibres should correlate with handedness. Our preliminary analysis of the pattern of lateralization showed a left lateralization of the hand U-shaped fibres whereas the dorsal and ventral fibres were symmetrically distributed. The left lateralization may be related to manual dexterity.

Future studies are needed to correlate lateralization of the hand U-shaped fibres with handedness, and the volume of mouth-tongue region with articulatory abilities. Comparative anatomical observations could reveal evolutionary modification of the U-shaped fibres and confirm their role in facilitating the development of complex movements of finger and tongue linked to the emergence of skillful object manipulation and vocalization in the history of human evolution.

4.2. FAT and premotor connections

The posterior regions of the superior and inferior frontal gyri are directly interconnected by a bilateral intralobar tract that

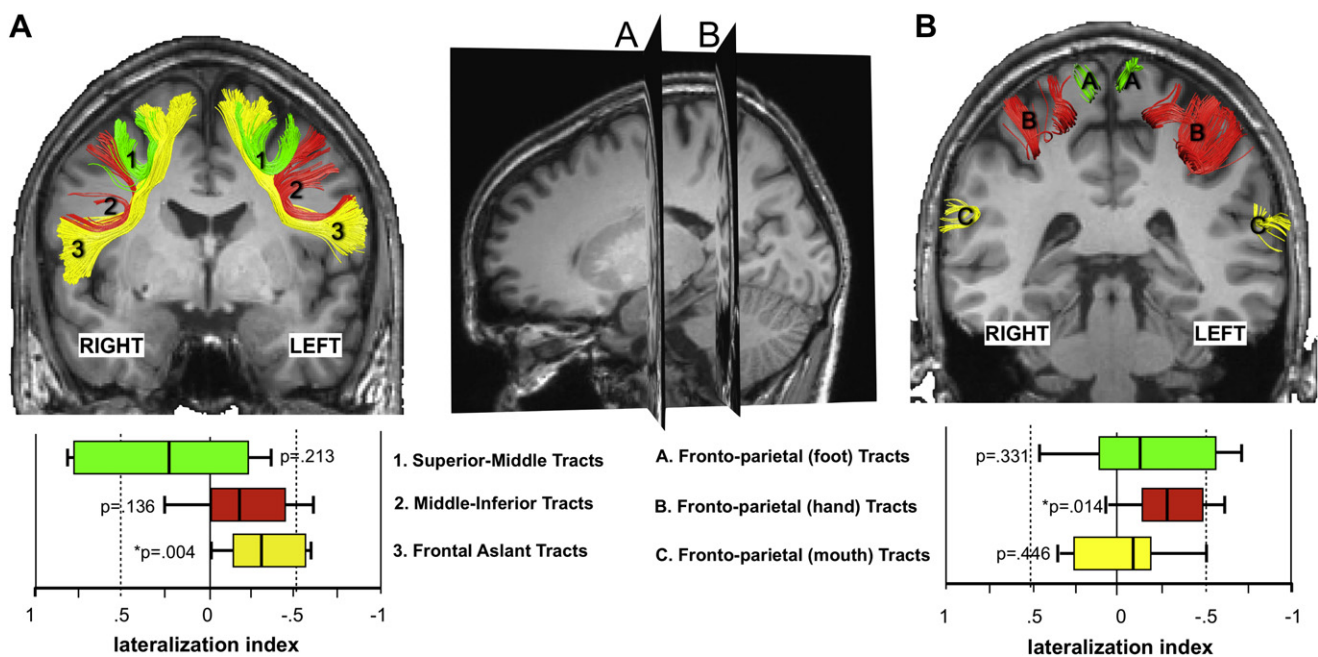


Fig. 9 – Analysis of the lateralization of the tract volumes. A) A statistically significant leftward asymmetry was found for the volume of the frontal aslant tract (FAT) (yellow) but not for the connections of the middle frontal gyrus with the superior (1) and inferior (2) frontal gyri. **B)** Similarly a statistically significant asymmetry was found only for the fronto-parietal U-tracts connecting pre-central and post-central gyri of the hand region (red).

has been previously described in the human brain using tractography (Oishi et al., 2008; Lawes et al., 2008; Ford et al., 2010; Guevara et al., 2011) and post-mortem dissections (Lawes et al., 2008). This tract has an oblique course from the medial-superior to the inferior-lateral region and for this reason we have coined the term frontal ‘aslant’ tract. Similar connections have been described for more anterior regions of the frontal lobe (Guevara et al., 2011; Ford et al., 2010) but we have not been able to dissect these components in our in vivo and post-mortem samples. These differences could be related to the methodological approaches used for the elaboration of the diffusion datasets (e.g., probabilistic vs deterministic tractography) or the selection of the regions of interest (e.g., automatic vs manual clustering) as well as the quality and the resolution of the acquired diffusion data.

In our study we also found that the superior and inferior frontal gyri are also connected to the posterior MFG through short U-shaped fibres. In turn each of the three regions connects to the striatum through descending projection fibres. The functions of the cortico-cortical connections of these regions are largely unknown. The medial projections of the FAT reach the anterior supplementary and pre-supplementary areas. Stimulation of these regions produce synergic movements of the eyes, head and arms ‘as though the individual were looking at the hand’ (Penfield and Rasmussen, 1950). Furthermore both medial-superior frontal and superior middle frontal gyri are part of a network for gaze control (Anderson et al., 2011). We speculate that the U-shaped connections between these two frontal gyri are part of an extended network involved in initiating and coordinating complex eye, head and arm movements for reaching actions.

This model could also explain the phenomenon of the ‘anarchic hand’ (i.e., a hand that produces unwanted movements interfering with the desired actions), which has been interpreted as an imbalance between the activity of the supplementary motor area (SMA), responsible for inner-driven actions and for the inhibition of automatic responses, and the lateral premotor cortex responsible for generating movements in response to external stimuli (Goldberg, 1985; Della Sala and Marchetti, 2005). The normal coordination between medial (SMA) and lateral (premotor) frontal activity mediated by the dorsal U-shaped tract is disrupted in patients with ‘anarchic hand’ where the damage to the SMA results in the inability to inhibit the automatic provoked responses (Mushiake et al., 1991; Della Sala and Marchetti, 2005). Similarly ‘utilisation behaviour’ (i.e., a compulsive urge to utilise objects at sight with either hand) (Lhermitte, 1983), often associated with bilateral SMA lesions, has been interpreted as an imbalance between intact premotor cortices, responsive to environmental triggers, and damaged SMA unable to inhibit inappropriate actions (Della Sala and Marchetti, 2005).

Stimulation of the SMA and pre-SMA also produces both vocalization and arrest of speech (Penfield and Rasmussen, 1950). Patients with lesions of the SMA and pre-SMA present various degrees of speech impairment from a total inability to initiate speech (i.e., mutism) to altered fluency due to ‘stuttering’ and monotonous intonation (Ackermann and Riecker, 2011). Most likely these medial regions of the SFG facilitate speech initiation through direct connections to the pars opercularis and triangularis of the IFG. We were also able to visualize connections between the dorsolateral and medial

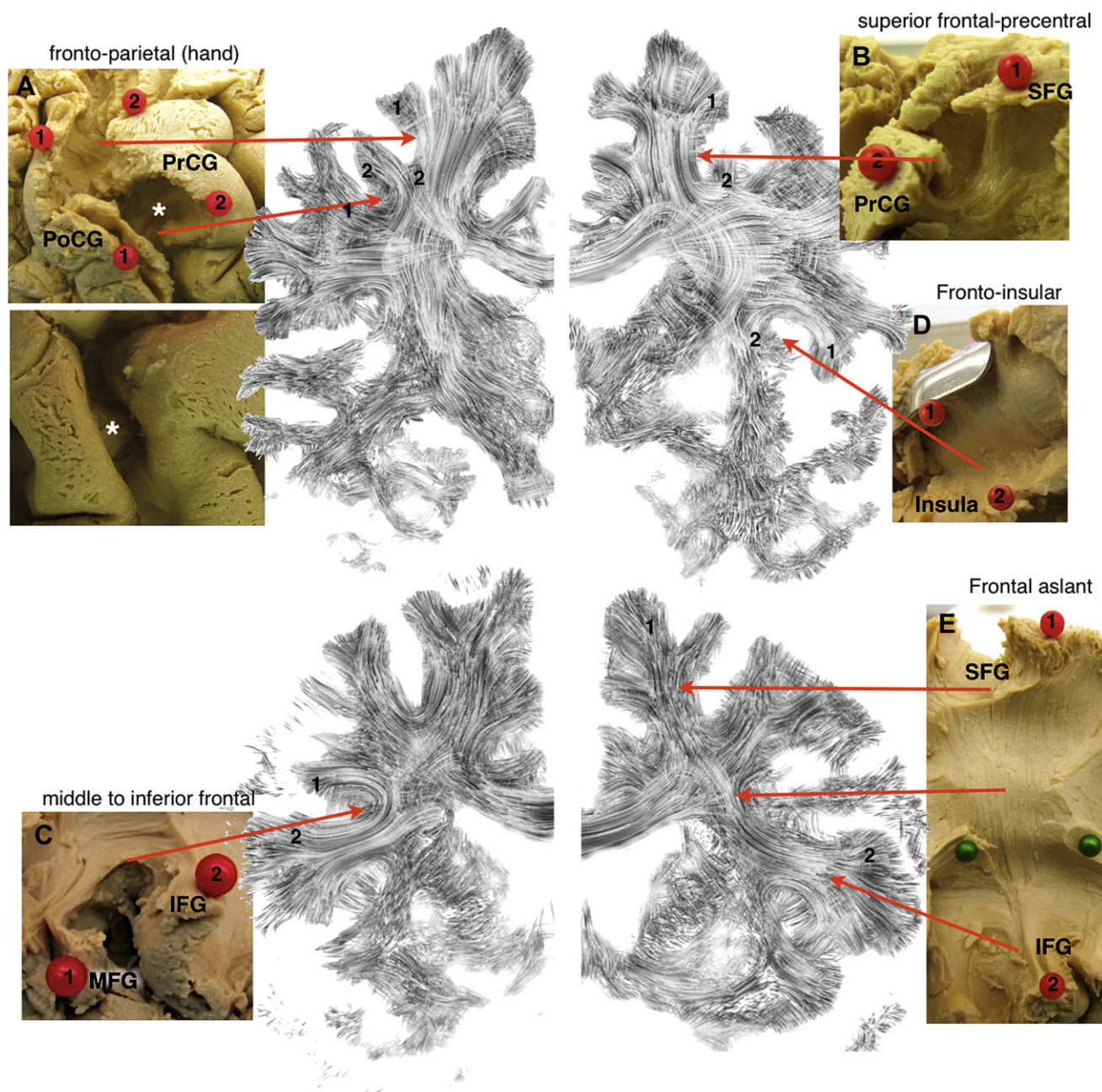


Fig. 10 – Coronal slices of the ‘Digital Dejerine’ maps and post-mortem blunt dissections of the corresponding tracts. **A)** Fronto-parietal U-tracts of the hand region connecting precentral gyrus (PrCG) with post-central gyrus (PoCG). The asterisks in the top left images indicate the correspondence between the presence of protuberances from the wall of the central sulcus and the underlying U-shaped tract. **B)** U-shaped tracts connecting the PrCG with superior frontal gyrus (SFG). **C)** Connections between the middle frontal gyrus (MFG) and inferior frontal gyrus (IFG). **D)** Fronto-insular connections. **E)** The frontal aslant tract (FAT) connecting inferior and superior frontal gyri.

cortical areas and the striatum. These direct connections indicate that cortical areas of the premotor regions rely also on an extensive subcortical loop for the initiation, coordination and performance monitoring of speech and complex limb movements (Fig. 8). Functional imaging studies also suggest that connections between the IFG and the caudate nucleus coordinate activities related to syntactic processing (e.g., recognition of sentences with incorrect sequence of words) (Moro et al., 2001). These activations are left lateralized in

most of the right-handed subjects. This functional lateralization could be related to the anatomical lateralization of the FAT. Furthermore the frequent observation of impaired fluency (Naeser et al., 1989), agrammatism (i.e., impaired syntactic processing) (Alexander et al., 1987) and reduced performance monitoring (Hogan et al., 2006) in patients with deep lesions in the periventricular white matter of the frontal lobes could be explained as a disconnection of the frontal aslant and fronto-striatal tracts.

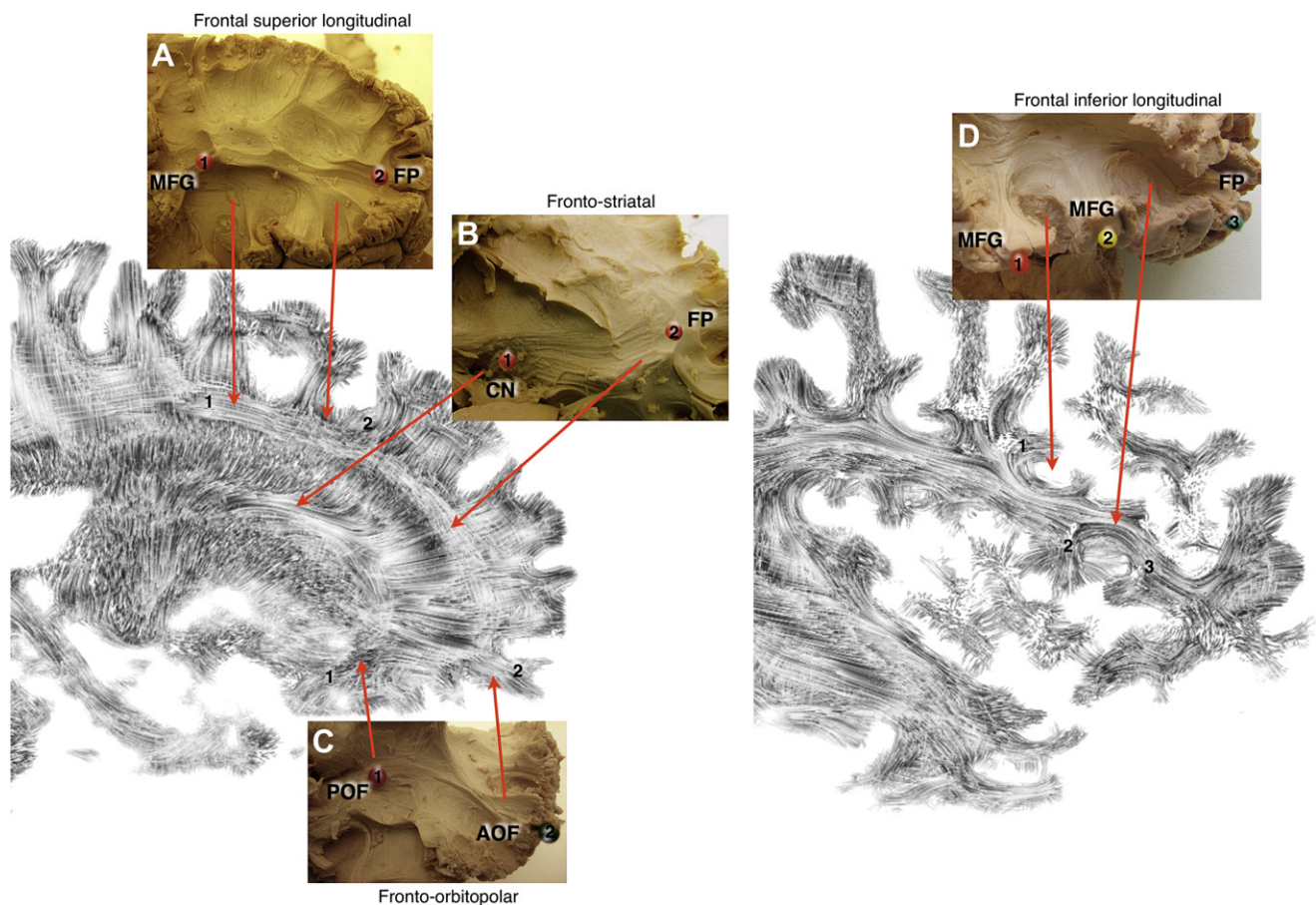


Fig. 11 – Sagittal slices of the ‘Digital Dejerine’ maps and post-mortem blunt dissections of the A) frontal superior longitudinal (FSL) system, B) fronto-striatal connections, C) fronto-orbitopolar, and D) frontal inferior longitudinal (FIL) system. CN, caudate nucleus; FP, frontal pole; AOF, anterior orbito-frontal; POS, posterior orbitofrontal; MFG, middle frontal gyrus.

4.3. FOP and FMT

The FOP is the main associative pathway between posterior orbitofrontal cortex and anterior orbitofrontal and polar regions. Similar connections have been described in the monkey brain between areas 10 and 13 (Price, 2007; Yeterian et al., 2012; Thiebaut de Schotten et al., 2012).

The posterior orbital gyrus receives inputs from the limbic regions (i.e., amygdala, hippocampus, nucleus basalis of Meynert, olfactory cortex and insula) and plays an important role in processing olfactory and gustatory inputs and integration of emotions and memories associated with the sensory experience (Rolls, 2002). The anterior orbitofrontal cortex receives auditory and visual inputs from posterior occipital and temporal cortex through the inferior fronto-occipital and uncinate fasciculus (Fig. 6) (Rolls, 2002; Price, 2007; Thiebaut de Schotten et al., 2011a, b). We suggest that the FOP represents a transmodal network for binding memories and emotions with olfactory, taste, visual and auditory inputs. This multi-sensory association and limbic integration could guide more complex cognitive and behavioural functions, such as reward behaviour associated with sensory and abstract reinforcers (e.g., monetary gain and loss) (Kringelbach, 2005) or response inhibition (e.g., go-no-go tasks) (Iversen and Mishkin, 1970).

In humans lesions to the orbitofrontal cortex manifest with a wide range of changes in comportment, such as lack of concern for the present or future, reckless behaviour, altered social manners, and disinhibition (Rolls, 2002; Zappala' et al., 2012). These patients fail to modify their behaviour on tasks that require changes of strategy in response to changes in environmental reinforcement contingencies (e.g., Wisconsin Card Sorting Test or Iowa Gambling Task) (Bechara et al., 2000). The correlation between the scores on the neuropsychological tests and the severity of behavioural symptoms in their everyday life suggests a common underlying mechanism for both cognitive and behavioural deficits in patients with orbitofrontal lesions (Rolls, 2002).

Our dissections of the frontal pole identified a prominent U-shaped bundle that we named the FMT for its course beneath the groove of fronto-marginal sulcus. The frontal pole is part of the prefrontal region, which corresponds to BA10. It is difficult to identify an equivalent of this area in the monkey brain but in humans it has certainly become the largest area of the prefrontal cortex (Semendeferi et al., 2001; Petrides et al., 2012). The prefrontal cortex is involved in working memory, episodic memory retrieval, mentalizing (Gilbert et al., 2006), monitor self-generated choices (Christoff et al., 2003), allocating attention between simultaneous tasks (Koechlin et al., 1999) and

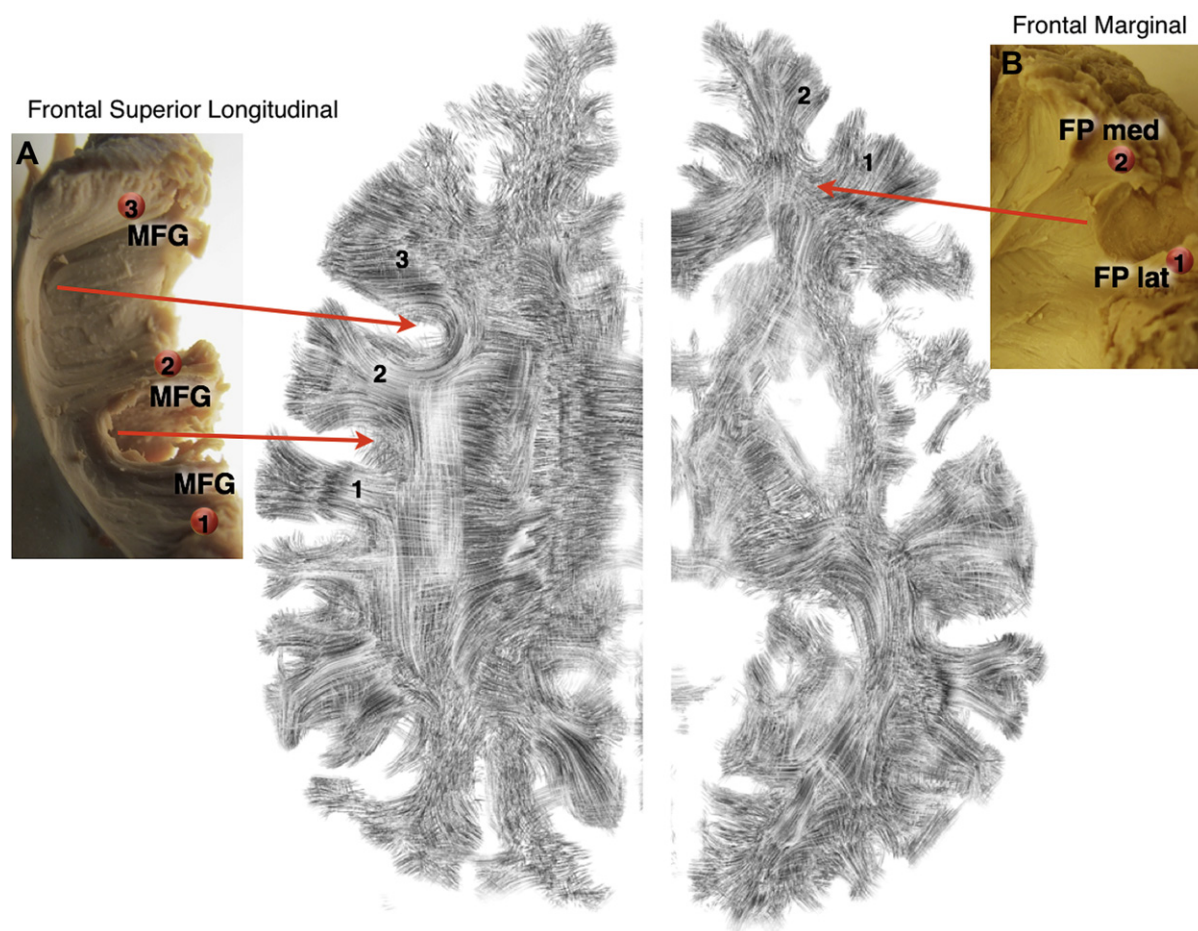


Fig. 12 – Axial slices of the ‘Digital Dejerine’ maps and post-mortem blunt dissections of the A) frontal superior longitudinal system (FSL) system and B) fronto-marginal tract (FMT) connecting lateral and medial regions of the frontal pole (FP).

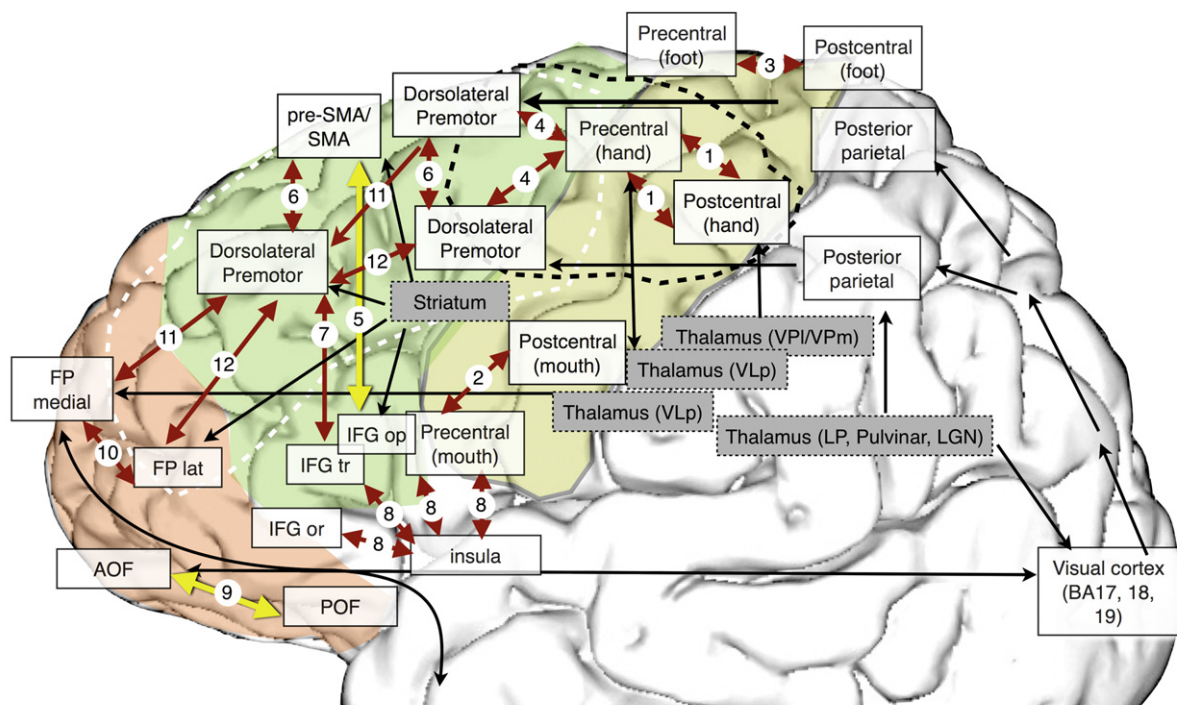
prospectively coding and deferring goals in multitasking (Burgess, 2000; Koehlin et al., 1999; Volle et al., 2011). Functional neuroimaging and lesion studies suggest that the lateral region of the frontal pole is particularly sensitive to working memory, episodic memory retrieval and attending environmental stimuli, whereas the medial frontal pole region is engaged in self-generated stimuli (i.e., the ‘thoughts in our head’) (Burgess et al., 2007). In particular in the context of the ‘gateway hypothesis’ proposed by Burgess et al. (2007) the role of area 10 is to determine whether signals from the internal (mental) or external (sensory) world dominate ongoing behaviour and cognition.

We hypothesise that the activity of the lateral subdivision of the area 10 depends on a three-component network (Fig. 6):

- i) The medial and ventral regions of the frontal pole have reciprocal connections with the thalamus, occipital extrastriate cortex and temporal cortex. These direct connections are probably bidirectional and subserve fast forward access of sensory information to anterior frontal cortex and top-down modulation of early perceptual processes. We speculate that these connections are involved in the encoding and retrieval phase of episodic memory tasks and other tasks involving feedback information (Tsujimoto et al., 2011).
- ii) The most rostrolateral cortex of the polar region connects to the striatum and the posterior lateral regions of the motor cortex. This network is likely to play a role in the planning and execution phase of working memory tasks, which involves response selection and monitoring.
- iii) The FMT may represent the anatomical link between these ventromedial and rostrolateral regions of the frontal pole.

4.4. FITs

We found a posterior to anterior pattern of distribution of the fronto-insular connections which replicates previous monkey (Mesulam and Mufson, 1985; Yeterian et al., 2012) and human studies (Cerliani et al., 2011). The subcentral region (BA43) is involved in sensory representation of the mouth and taste perception and can be considered an extension of the primary somatosensory cortex. This the only region of the frontal lobe that shows connections to the posterior insula. The other short fronto-insular connections establish direct communication only with the anterior insula (Fig. 7). The anterior insula receives visceral and sensory (especially gustatory and olfactory) input and integrates it with limbic motivational-emotional afferents. It also sends efferents to subcortical structures involved in alimentary functions (e.g., salivation, gastric



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|--|--|---|
| ① Fronto-Parietal U-Tracts (hand) (Figures 3, 4) | ⑤ Frontal Aslant Tract (Figure 5) | ⑨ Fronto-Orbitopolar Tract (Figure 6) |
| ② Fronto-Parietal U-Tracts (mouth) (Figure 3) | ⑥ Fronto Superior-Middle U-Tracts (Figure 5) | ⑩ Fronto-Marginal U-Tract (Figure 6) |
| ③ Fronto-Parietal U-Tracts (foot) (Figure 3) | ⑦ Fronto Inferior-Middle U-Tracts (Figure 5) | ⑪ Frontal Superior Longitudinal Tracts (Figure 8) |
| ④ Fronto-Precentral-U-Tracts (Figure 4) | ⑧ Fronto Insular U-Tracts (Figure 7) | ⑫ Frontal Inferior Longitudinal Tracts (Figure 8) |

Fig. 13 – Diagram of the frontal lobe connections. U-tracts are in red, intralobar frontal tracts are in yellow and the long-range association and projection connections are in black. The different areas outlined correspond to the different functional divisions as following: central sulcus connections (yellow area), hand-knob connections (dashed black line area), premotor connections (green area), prefrontal and orbito-polar (light red area), dorsolateral longitudinal connections (dashed white line area). AOF, anterior orbitofrontal; FP, frontal pole; IFGop, pars opercularis; IFGtr, inferior frontal gyrus pars triangularis; IFGor, inferior frontal gyrus pars orbitalis; LGN, lateral geniculate nucleus; LP, lateroposterior nucleus; POF, posterior orbitofrontal; SMA, supplementary motor area; VLp, ventral lateral posterior nucleus; VPI, ventral posterior lateral; VPm, ventral posterior medial.

motility). The frontal operculum controls orofacial movements required for non-verbal facial expression, mastication and deglutition, speech articulation, prosody and syntactic and semantic aspects of language. Hence, direct insular inputs to these posterior frontal regions could, for example, provide visceral and emotional information to modulate speech output according to internal states. Conversely frontal projections to the insula could trigger salivation and gastrointestinal motility associated with mastication and swallowing.

Stimulation and lesion studies suggest a functional and anatomical segregation of the FITs (Penfield and Rasmussen, 1950; Mesulam and Mufson, 1985; Dronkers, 1996; Ackermann and Riecker, 2011; Nestor et al., 2003; Augustine, 1996). We speculate that posterior connections between pre-central (BA4 and 6) and insula are involved in integration of taste and visceral (e.g., epigastric discomfort and nausea) sensation with movements associated with mastication, vomiting and facial expression. Connections to the pars opercularis (BA44) of the IFG are likely to be involved in speech articulation, vocalization of emotional states and facial expression. Lesions to these insular connections may result in orofacial and speech apraxia (Dronkers, 1996), flat intonation (motor aprosodia), dysphagia

and vomiting. More anterior insular connections to pars triangularis (BA45) and orbitalis (BA47) of the IFG are probably involved in semantic and memory functions associated with taste, visceral sensation and emotions. Lesions to these connections can produce deficits in semantic memory and verbal fluency (Bates et al., 2003; Nestor et al., 2003). It is likely that the insula connections to the different regions of the inferior frontal lobe have some degree of anatomical and functional overlap which we were not able to visualize with our method.

4.5. FLS

The FLS consists of two parallel chains of U-shaped tracts connecting motor, premotor and prefrontal regions. It is composed of a dorsal FSL tract coursing beneath the superior frontal sulcus and a ventral FIL tract running in close proximity of the inferior frontal sulcus. The FLS represents an extension of the superior longitudinal fasciculus connecting fronto-parietal regions (Thiebaut de Schotten et al., 2012). The superior longitudinal fasciculus is composed of three branches, whereas only two chains of connections form the FLS we observed in the frontal lobes. It is possible that this is

related to the limitations of the tractography algorithm that fails to reconstruct small U-shaped connections of the most dorsal region (false negative).

The functions of some of the connections of the FSL tract have been described in the context of the motor premotor connections of the hand-knob region. In general the FLS is likely to play a role in integrating the activity of the different local networks of the frontal lobe, such as, for example coordinating movement planning and execution (carried out by the motor and premotor networks) with an overall goal directed strategy supervised by the FP networks. Lesions to the FLS are likely to manifest with impairment in executive functions, sustained attention and working memory (Stuss et al., 2002; Grafman, 2002).

5. Conclusions

In this study we attempted to visualize the intralobar network of the human frontal lobes. The use of spherical deconvolution and post-mortem dissections is a valid approach to overcome some of the limitations derived from axonal tracing studies and classical tensor based tractography. It remains to ascertain whether the representation of some of the tracts is biased by the presence of merging fibres (e.g., callosal) connecting to the same cortical regions of the frontal lobe (Berlucchi, 2012). Furthermore our dissections were performed only on two subjects and need confirmation in a group study. Nevertheless these preliminary findings can be used as framework for understanding heterogeneity of the anatomy of these pathways in larger groups of subjects and correlate their anatomy with cognitive and behavioural performances in healthy population and brain disorders. Future studies are needed to compare these results with other complementary methods that could shed light on some of the functional correlates of these tracts (Matsumoto et al., 2011; Duffau, 2011; Duffau, 2012).

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